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What (if any) future for nuclear power?

WHAT on earth is to be done about the nuclear power industry? For the past several years, governments in the industrialised West have been perplexed to know how best to handle the domestic problems in which their own industries have become enmeshed. In most places there is a groundswell of public opposition, or at least of disquiet at the prospect that large numbers of nuclear power stations may be built. Elsewhere there are more specific problems to be resolved. In the United States, for example, the shadow of the Three Mile Island accident has not been fully dispelled by the Kemeny Report. The Nuclear Regulatory Commission has been reorganised but the 7 June deadline for the licensing of reactors built but not yet operating has been allowed to slip, postponing the time when eight reactors now ready to generate electricity can be put into commission. In the United Kingdom, the immediate problems are different but no less daunting — after a decade of optimism and two decades of hesitancy, the doubts of the public authorities (the government and the nationalised utility) about the kinds of reactors they would like to build are matched only by more general doubts about the capacity of the construction companies to do their part of the job. What (if anything) is to be done?

Perhaps the first need is to acknowledge that the present uncertainty about the future role of nuclear power is shot through with irony. First, uncertainty about nuclear power coincides with that period in recent industrial history at which a successful nuclear power industry would be as welcome as the prophets of the 1950s were fond of promising. Second, the industry itself, once fond of explaining to anybody willing to listen that its technical competence was so easily demonstrable, and its technique so innovative, that by its example it would show how the rest of industry might join the Twentieth Century, now finds itself pilloried for incompetence.

The President's Commission on the Three Mile Island accident could and should have been much more critical of the technical design of the reactor that went wrong. (It is almost beyond belief that thermocouples in the reactor core were designed to produce temperature readings within the normal range and otherwise merely question marks.) In Britain, the Advanced Gas Cooled reactors, conceived unnecessarily in the early 1960s, have been painfully and unprofitably slow in gestation. Third, public opinion of nuclear power, once approving and even applauding, has turned sour to say the least. The newspapers that were once chauvinistically full of tales of how clever their nuclear engineers had been are now full of tales, even fairy tales, of how dangerous nuclear power may turn out to be.

It is a matter of great importance, for the professional community as well as for the public at large, that these dilemmas should be quickly and sensibly got rid of. For the reputation of science and technology will be permanently tarnished if the muddle about nuclear power persists much longer. Indeed, there is a sense in which any resolution would be better than none. Not merely does the present hesitation keep large numbers of skilled people at work on plans that may never come to pass, which is a waste of resources and dispiriting for those concerned. It is also bound to seem a sign, to many intending entrants to these professions, that science and technology are much less able to change the world in welcome ways than they would have had reason, in the 1950s at least, to hope. Altruism, to be sure, is only a part of the reason why young people make careers in one field or another, and in any case there are many other fields of science and technology where

people can hope to work in ways that are rewarding for others as well as themselves. It would however be mistaken to shrug off the disappointments of the nuclear power industry as entirely irrelevant.

Moreover, it is not simply bad luck that worldwide hesitancy about nuclear power should come at a time when oil is in short supply. Raking over the past, always entertaining and sometimes instructive, is also frequently dispiriting. It is however important that many of the present problems of the nuclear power industry stem from a misreading of the problems of energy supply nearly a quarter of a century ago. Thus in 1957 the OECD (then the OEEC) published a report on energy prospects by a committee under the late Sir Harold Hartley which, in essence, urged on Western governments that the most economical way of generating electricity would be by the use of cheap and plentiful oil from the Middle East. Many governments, conspicuously the then British government, took that as an opportunity for cutting back on nuclear developments. In retrospect, it is almost inexplicable that in 1970 a task force appointed by the then President Richard M Nixon assured the White House that supplies of imported oil would be reliable and free from the risk of political interruption. In reality, recent events in the oil markets of the world have been on the cards since the formation of OPEC in the early 1960s. It is too late now to know whether the course of OPEC prices would have been different if the industrialised oil importers had prosecuted the development of nuclear power more vigorously. But shortsightedness is a more plausible explanation for the coincidence of crises than bad luck.

The way in which the reputation of the nuclear power industry has been tarnished with the passage of the years is also not an accident. Again in retrospect, it is quite breathtaking that the plans hatched in the 1950s by the then new nuclear industries of the West should have been as ambitious — and as brash — as events have shown them to be. The British government's White Paper on the subject, published in 1955, will surely rank high in the archives of official wishful thinking, with its plan for embarking on a dozen nuclear power stations, involving no fewer than four different designs, in a mere decade. During the same period, nuclear people underestimated the problems such as long-term waste disposal, the risk of reactor accidents (and of the steps that should be taken to deal with them). The fact that some reactors (Three Mile Island) and some reactor types (Advanced Gas-Cooled reactors) have not lived up to what was expected of them has inevitably sharpened public uneasiness about the future of nuclear power. It must, of course, be acknowledged that opinion is also divided within the professional community.

In such circumstances, it is unreasonable and even unwise to ask that governments should not be hesitant. What else can democratic governments do but balance the conflicting wishes of their electors? To ask that they should ram disputed policies down the throats of a divided electorate is to ask that they should cease to be democratic. In Britain, if the protracted examination (now under way) of the government's new ten-year nuclear programme by the House of Commons Select Committee on Energy will help to reconcile conflicting opinions, the time spent will have been worthwhile. The promised public inquiry on the proposal to build a single pressurized water reactor may also help, at the cost of further delay. No doubt the Kemeny Commission's report will in due course also be a kind of balm. None of this, of course, excuses those occasions on which governments have timorously



postponed decisions that should have been taken (the previous British government), have over-reacted to public anxiety about some aspect of nuclear power (President Carter's pointless Non-Proliferation Act (see page 526)) and have tolerated incompetent administration (that of the US Nuclear Regulatory Commission before Three Mile Island). The consequence, however, is that it will now be several years before the industrialized nations can hope to reap whatever benefits there may be in fully integrated fission fuel cycles. In Britain, for example, it will not now be possible to commission a full-scale fast reactor before 1995.

Many professional people are deeply frustrated by this prospect. Many others, and a large section of the community at large, are fearful that even 1995 is too soon for comfort. What is to be done to reconcile these views? Given the unavoidable limitations on what elected governments can set out to do, the long-term goal must be a more or less common public understanding of what the hazards of nuclear power may be. But how, some will say, can that be attained when the technical issues are so complicated, involving the probabilistic assessment of unfamiliar risks, attempts to estimate what happens over centuries to radioactive wastes and esoteric bits and pieces of high technology? Fortunately, this is a travesty of the potential for public understanding. First, there is no reason why the public at large should not come to as useful an understanding of the niceties of nuclear power as it has, in the past decade, of the relationship between increases of salary and the rate of inflation. Second, public confusion mirrors the division of professional opinion. Only that sustains the crowds willing to turn out on public holidays for this

or that demonstration against nuclear developments of any kind, but the division is troubling also for those who stay at home.

The key to the future of nuclear power therefore rests where it belongs, with the professional community whose reputation is, at least in part, at stake. What must be done to bridge the gulf that now divides it? Pure reason will not by itself suffice, people's positions have become deeply entrenched. Those who hold that even fast reactors can be built without unacceptable risks have a duty to explain themselves to their colleagues (which, fortunately, they have recently been more inclined to do). Those who hold that long-lived waste can be disposed of (or stored) safely for periods of the order of 10,000 years must say how (or otherwise think of something better, like the separation of the actinides from high-level waste). Similarly, those who argue that the risks and dangers of nuclear proliferation are proportional to the amount of plutonium manufactured must be asked to justify their arithmetic. Those who hold that the risks of nuclear power are inherently greater than those of other enterprises must explain why their opinion is not cant. The trouble with the nuclear debate within the professional community is that, in spite of all the abuse that has been thrown about, it has been excessively polite. People have joined advisory committees to help write predictable reports, people have made speeches, given evidence to inquiries and talked to the newspapers and have considered their duty done. The important need is that professional people should convince each other.

More trouble about students' fees

MR Mark Carlisle, Secretary of State for Education and Science, managed only a second-rate defence of his government's policy on fees for overseas students in the House of Commons on 5 June. Although a more skillful parliamentarian might have shown his critics a cleaner pair of heels, Mr Carlisle's difficulties stem from more basic troubles. For the policies he and his colleagues are following are both inconsistent and unrelated to their other concerns, such as they may be, for the development of higher education in the United Kingdom. They will discover their defence of their position just as difficult until they are able to work out among themselves a more convincing explanation of what they are about. In the meantime, higher education and the universities in particular, not to mention their prospective students, will be subjected to needless uncertainty and, perhaps, damaging financial trouble.

Although there are many British academics who still hanker after the principle that all students at the same university should pay the same tuition fees, this was abandoned by British universities, at the government's request, more than a decade ago. And, indeed, there is something in the view that students who do not themselves or through their families contribute towards the cost of a maintained university system should pay somewhat higher tuition fees than others. This is, of course, the basis on which the state universities in the United States operate systems of tuition fees that differentiate between students from within and from outside the state concerned. That some countries, France for example, make no distinction between the fees of home and overseas students is logically irrelevant even though it may (and should) be a reminder of the value often attached to the principle of open access to higher education. The issue which has divided academics in Britain from their paymasters in the government is, however, the pragmatic question of what fees should be charged to students from overseas.

It is only charitable to suppose that when the British government announced just over a year ago that it would seek to recover the 'full economic cost' of educating overseas students, it had no more than a calculation on the back of an envelope to help. In the event, the government has interpreted that as the average cost of teaching students, implying that new students will be charged tuition fees comparable with even those in North

American universities from the beginning of the academic year. Last week, Mr Carlisle had no reply to the argument that marginal cost would have been a better basis.

For the time being, indeed, the government seems to be simply hoping for the best. All along, the universities have been concerned that the high cost of the fees now in prospect would so effectively frighten away new students that their total incomes might actually decline. (The government plans to enforce its will by reducing the recurrent grants the universities will receive in future years in proportion to their populations of overseas students.) Last week, Mr Carlisle made as much as he could in the House of Commons of the fact that the returns from the Universities' Central Council on Admissions are only 12 per cent below the corresponding figures this time last year. This, however, is an insubstantial comfort (see *Nature*, 27 March). Many students who have applied for places next year will not turn up. Others will turn up without their full fees. No amount of public speaking at this stage can discover what will be the consequences next September.

Inevitably, however, some universities and polytechnics will be more seriously affected than others. If overseas students were to show the same preferences among universities as do British students, the result might even be to take resources away from those institutions that are least able to attract students of any kind. There are grounds for hoping that in due course British universities will indeed be helped to be more competitive among themselves (*Nature*, 22 May). Unfortunately, the perturbation caused by the higher fees now in prospect is that individual universities will be affected in a more or less random fashion, according to their present intake of overseas students. If the government has seriously embarked on the encouragement of a free market in higher education, there are several constructive steps that it could take. Already, students' fees account for more than a fifth of universities' incomes, more than twice the proportion of fees obtaining before the last large increase of all students' fees in 1977. The degree of universities' dependence on the University Grants Committee's continuing support is in other words already declining. A further arbitrary push in this direction is not what the system now needs. Why not, even at this late stage, settle for marginal costing as a basis for calculating all fees?

Administration comes in from the sun

Washington

The Carter Administration's image as a strong supporter of solar energy is going into eclipse. Last June the President committed the United States to the objective that 20 per cent of its energy should come from the sun by the year 2000; but the Administration is already discussing budget figures for solar research and development which, critics say, make this goal unachievable.

The source of the controversy is a memorandum leaked from the Department of Energy last month revealing current thinking on the evolution of the energy budget over the next five years. This shows an increasing share for nuclear and fossil energy — from 37 per cent of the budget in 1981 to 43 per cent in the period 1982-86 — and a corresponding drop from 24 to 20 per cent for solar energy and conservation efforts.

Defending these figures in front of a congressional committee last week, Energy Secretary Mr Charles Duncan pointed out that they were not firm commitments, but were intended as background to preparations for the 1982 budget request, and were likely to alter from year to year.

"I believe we are on track toward achieving the nation's solar goals", Mr Duncan said. "But I believe it is unrealistic to assume, as some do, that we can lay out a detailed and precise road-map that tells us what is going to happen year by year for the next twenty years."

Given the vagaries of technological forecasting, Mr Duncan's logic is not disputed. But according to published and unpublished documents produced by committee chairman Representative Richard Ottinger, at least some DoE officials and their outside advisers feel there is a growing gulf between the political rhetoric surrounding long-term goals, and the political realities of short-term budget choices.

At the centre of the dispute is President Carter's 20 per cent target, itself based on a study carried out by White House staff and the DoE. Presented with various policy options, the President selected the "maximum practical goal" of 18.5 quads of solar energy by 2000, when total energy demand is expected to reach 95 to 100 quads. Politically, this was judged the least that the vociferous solar lobby, which had pushed for an even larger commitment, would accept, and the most that sceptics within the Administration could live with.

The President admitted that achieving the goal would require not only substantial cooperation from the private sector, but also a vigorous and sustained commitment by the federal government.

It is the desirability of this federal involvement that has since been challenged, particularly in a period of budget stringencies and of pressure to

reduce public participation in potentially commercial programmes.

Earlier this year, for example, the Committee on Nuclear and Alternative Energy Systems of the National Academy of Sciences aroused the anger of solar advocates with a report claiming that the President's goals could be approached only by federal subsidies far higher than those currently given to other energy sources. The issue has been sharpened by the tightening of budget constraints.

The detailed expenditure proposals in last year's White House review are substantially higher than those being considered by the DoE. Thus, the policy review suggested that biomass could be contributing 5.4 quads a year by the end of the century, but that an investment of at least \$150 million a year over the next five years would be needed. In contrast, the DoE estimates reckon on a more modest budget of \$60 million a year over this period.

What angers the critics most, however, is not so much the precise levels of proposed funding as broader evidence that the Department of Energy may not be giving adequate attention to the solar and conservation fields.

Here they are supported by two recent reports from congressional review agencies, the General Accounting Office and the Office of Technology Assessment, both of which take the DoE to task for failing to take the steps necessary to

implement the Administration's solar goals.

The OTA report, for example, published in Washington last week, concludes that solar and conservation programmes are hampered by a lack of direction and leadership.

Energy Secretary Duncan admitted at last week's hearing that such management problems had existed in the past, but listed recent steps he had taken to resolve them.

Duncan also listed the Administration's achievements in solar energy, quoting the \$36 million recently awarded for the installation of solar heating and cooling systems in federal buildings, and contracts for nine major photovoltaic systems in commercial and industrial settings.

Solar advocates are not overly impressed. They point out that, although the Administration can demonstrate a spectacular rise in support for solar energy from a few million dollars in the early 1970s to a proposed budget of \$1,400 million in 1981, Congress has led the way.

For two years things looked different, with positive discrimination apparently flowing from the White House in favour of solar energy as an alternative to less environmentally-acceptable options. Last month's leak may result in a small increase in the solar budget request when it comes formally to Congress next January. Otherwise, it looks as if it will be business as usual.

David Dickson

Polish chemist gets off lightly

Mirosław Chojecki, a young Polish chemist and campaigner for academic freedom, last week went on trial in what Polish intellectual circles regard as a test case — and received as near as the authorities could come to an acquittal.

Chojecki was formerly employed at the Swierk nuclear research centre near Warsaw. In 1976, he became a member of the "Workers' Defence Committee" (an unofficial human rights group), and shortly afterwards was dismissed from his job. Since then, he said before his trial last week, he has been allowed no access to any scientific libraries or periodicals. With his own academic career at an end, Chojecki and a few friends founded, in 1977, the "Independent Publishing Enterprise, NOWA", which strives to fill the gap caused by what he described as the state monopoly of information.

In particular, NOWA has published several textbooks for the underground "Flying University" — a dissident educational self-help body which, although specialising in the social sciences and humanities, numbers among its patrons some of the most distinguished Polish natural scientists.

(Its "Dean", Professor Jan Kielanowski, is a member of the official Polish Academy of Sciences, and a world authority on animal nutrition.)

Not surprisingly, the "Flying University" came out strongly with an open letter in Chojecki's defence. Tacit support from the scientific community at large, said Chojecki, was even more widespread. On two occasions, leaflets calling for his acquittal were discharged over central Warsaw from a catapult mounted on the building of the Academy of Sciences itself.

The formal charges against Chojecki were misappropriation of a duplicator and incitement to induce two employees of a state printing concern to print a dissident work. In fact, as he stated in his 2000-word defence, everyone in court was aware that the whole issue of intellectual freedom was on trial. Chojecki made no effort to deny his publishing activities — he even submitted as evidence the catalogue of works he had produced. After an 11-hour hearing, he received an 18-months suspended sentence, and a fine which was waived in consideration of his pre-trial spell in prison.

Salaries

Physicists get on

EITHER go west, or work for the government — that seems to be the inference that young British physicists will make from the latest remuneration survey among members of the Institute of Physics published in the most recent issue of *Physics Bulletin* (May 1980).

The figures now published show that the salaries of physicists working in the Civil Service consistently exceed those of academics and industrial physicists once the age of 40 has been passed. In the age group 55-59, the peak of physicists' earnings, the median salaries of those in the institute's two most highly qualified categories of membership (fellows and members) are now £15,750 p.a. for civil servants, £13,060 for academics and £11,940 for industrial physicists. The survey has shown the same pattern of salary differentials for the institute's membership as a whole.

The results of the latest survey show that there has been no radical change in the pattern of physicists' salaries since earlier surveys in 1977 and 1974. Broadly speaking, young people do best by working in industry immediately after graduation. Fellows and members of the institute in the age group 25-29 had median salaries (in 1980) of £6,570 p.a., compared with £5,850 and £5,780 in government service and the universities respectively. For these categories of membership, the financial advantages of industrial life persist until 35, but in later age groups government physicists are ahead of those elsewhere.

Alternatively, the most highly qualified members of the institute can expect their salaries to increase during a working lifetime (between the age groups 25-30 and 55-59) from a median of £5,850 to a median of £15,750, a ratio of 2.9. The corresponding ratios for academics and those working in industry are respectively 2.26 and 1.82.

The latest survey thus bears out the suspicion of many of those leaving universities, in other fields of science as well as physics, that industrial employment, although initially somewhat more rewarding in financial terms, offers a less dynamic pattern of employment than government service and academic life.

The latest survey also shows, however, that physicists have managed to keep up with inflation reasonably well in the past few years. In all categories of membership, and in all age groups, salaries have more than doubled since 1974.

The largest increases have however gone to the younger age groups and to those in the least qualified categories of membership (associate members and associates). Indeed, the median salary of associates of the institute in the age group 25-30 (£6,720) now exceeds that of the members of the same age (£6,290 p.a.)

Proliferation

Trimming sails

Washington

More carrot, less stick. That is the message being given to President Carter by State Department officials as the US Administration reassesses its strategy for limiting the spread of nuclear weapons through unilateral controls on nuclear technology.

Staunch non-proliferationists in Congress and other parts of the Administration are demanding that the President stand firm, and in particular that he reject India's pending request for 39 tons of enriched uranium for its Tarapur power plant because of its refusal to accept international safeguards.

But elsewhere there is growing feeling that, in order to maintain credibility and effectiveness, the US must shift from the 'control and denial' aspects of non-proliferation strategy to emphasising the incentives for compliance offered by US promises of a 'reliable' supply of nuclear fuel and technology.

Such sentiments have risen to the surface following the completion in February of the International Nuclear Fuel Cycle Evaluation. Proposed by President Carter in 1977, INFCE endorsed several aspects of US policy — but fell far short of endorsing all of them.

Supporters of the nuclear industry are now using the INFCE findings to challenge key aspects of Carter's policies. For example the House of Representatives Commerce Committee last week recommended that the Nuclear Regulatory Commission proceed with licensing review procedures for a new reprocessing plant; these were suspended in 1977 at the President's request, with the promise that the order would be re-examined after the completion of INFCE.

Despite indications of a possible relaxation, the Administration has given no sign that it intends to change its basic policy in the light of INFCE. Rather it is seeking ways to enhance its image as a reliable supplier of nuclear technology, within the provisions of the Nuclear Non-Proliferation Act of 1978.

The problem is that the act poses what Dr George Rathjens, professor of political science at the Massachusetts Institute of Technology and deputy to Mr Smith, calls a 'fundamental dilemma'.

"Our policy and our law require that we condition our supply of fuel and technology on others accepting US approval rights over the use of US-origin materials", he told the congressional sub-committee. "Yet our imposition of conditions on supply necessarily reduces the confidence of others that they will have access to fuel and technology."

Various ideas on how to get round this difficulty in the light of the INFCE results are now being discussed. Drawing

on INFCE's conclusion that the economics of reprocessing appear to be marginal, for example, Mr Smith has proposed that the US agree to reprocessing by other countries wherever the demand for plutonium is dictated by the needs of fast breeder and advanced reactor research — but not where it is needed merely to manage spent fuel.

Other suggestions being pursued by the State Department are that longer-term licensing could replace current individual requests to transfer and reprocess spent fuel from states with good non-proliferation credentials, and various types of back-up fuel supply arrangements, perhaps with one country agreeing to meet commitments if another country defaults.

Whether changes in emphasis within the existing legislation will be sufficient to meet the objections of critics remains to be seen. Certainly the outcome of INFCE has done little to shift the reluctance of the member countries of Euratom to renegotiate its uranium supply agreements with the US, as required by the NNPA.

Similarly, even if Britain adopts a Westinghouse design for its next thermal reactors, the provisions of the act could lead it to reject Westinghouse as a supplier

Non-proliferation act

The Nuclear Non-Proliferation Act which was signed by President Carter in 1978 is an attempt to limit the proliferation of nuclear weapons by tightening restrictions on the supply of nuclear fuels and facilities by the US to foreign countries.

The act was prompted by India's explosion of a nuclear device in 1974 using nuclear fuel and technology initially provided by Canada for energy purposes. It forbids the supply of nuclear fuels, reactors or reactor components to countries not possessing nuclear weapons which explode nuclear test devices, or which otherwise violate safeguards developed by the international atomic energy authority.

A decision to refuse an export licence for such fuel or reactor components can, however, be overturned by the President if it is considered that such action would be "seriously prejudicial to the achievement of United States non-proliferation objectives or otherwise jeopardize the common defense and security." The President's action can in turn be vetoed by Congress.

In addition, the act provides that no nuclear fuel or technology exported from the US can be retransferred to the jurisdiction of any other nation or group of nations without the prior approval of the US. Nor can spent nuclear fuel either originating in the US or produced with technology from the US be reprocessed without US approval.

of major components, such as the reactor vessel and circulation pumps. Under the current terms of the act, the US would have control over any fuel once it had been irradiated using such components.

But opposition to a change in policy is firmly entrenched, particularly from those convinced of President Carter's correctness in opposing any measures that would increase the spread of plutonium. "The choice between us is not between 'leverage' and 'consensus', rather it is between sticking to our principles and abandoning them" says Dr Tom Cochran, senior staff scientist with the Natural Resources Defense Council.

Given the apparent depth of the President's previous commitment to this position, no substantial change of policy is expected, at least until after the forthcoming elections. But there are several hurdles to be met before then, in particular the Nuclear Non-Proliferation Treaty review conference which takes place in Geneva in August.

Faced with inevitable criticism for failing to secure progress towards an arms limitation agreement with the Soviet Union, the US is hoping to reply by pointing to its efforts in support of INFCE's warnings about the proliferation dangers inherent in reprocessing and fast breeders — and to the steps it is taking to enhance its image as a reliable nuclear supplier to states which sign the NPT.

But it is a long shot. US officials admit that they are faced with a no-win situation at the review conference, and that their prime tactic is likely to be 'damage limitation' rather than anything more ambitious.

David Dickson

Electric vehicles

Top speed at Lords

What will the House of Lords make of the electric car, the topic on which the Select Committee on Science and Technology appointed in January (*Nature* 20 March, page 199) chose to cut its teeth? Nobody is yet sure. But anxious, no doubt,

to fulfil its promise that the inquiry should be short and sharp, the committee has now finished taking evidence and plans to publish its report in July. Electric vehicles are in the same class as saints used to be: everybody approves provided they work. The perennial problem is whether the energy density of the batteries that drive electric vehicles can be increased substantially above that of the lead-acid battery, rated at 150 kilo-Joules kg⁻¹ at full charge.

The committee has heard from the chief British battery makers, Chloride and Lucas, that the brightest prospects for increasing energy densities by a factor of between three and four lie with the sodium-sulphur battery. Unfortunately though, vehicle operators would have to put up with the inconvenience of batteries working at 350°C. So nickel-zinc batteries now seem to be the gleam in manufacturers' eyes.

Several witnesses have told the House of Lords that Britain spends little on electric vehicle research compared with the United States and Japan. About £20 million has been spent since the late 1960s, about 75% of which has been put up by private industry. The US, by contrast, has a seven-year \$200 million programme which began in 1976. Yet Britain has the largest fleet of electric vehicles — 45,000 of them, mostly used for delivering milk.

The prospects for further and rapid growth of the electric vehicle fleet in the UK are not however bright. Chloride/Talbot and Lucas/Vauxhall have built small fleets of delivery vans (top speed 50 miles per hour and range 50 to 70 miles), which are now operating in several cities. They hope to achieve commercial production in the mid-1980s.

But the manufacturers are cool about the prospects for the all-electric private car. The most promising way of increasing speed and range is by means of the hybrid vehicle running on part petrol, part battery. US manufacturers seem to be much keener on this development than their UK counterparts who are deterred by the problems of incorporating two different systems within one vehicle. A

hybrid idea which has, however, been greeted enthusiastically in the UK is the hybrid trolley-bus, which runs partly from an overhead power supply and partly on batteries.

Potential operators, the House of Lords has heard, will have little to choose between electric and conventional vehicles in terms of the efficient use of primary fuel. Operating costs will, of course, depend on the relative prices of electricity and oil. But the efficiency of the electric vehicle in terms of the load it can carry can be considerably lower than that of the conventional van because of the great weight of its batteries.

Perhaps the most important question House of Lords committee members have had at the backs of their minds is whether the level of funding in the UK is sufficient, given the current state of knowledge. The Electric Vehicle Development Group, which exists to support and advise manufacturers interested in electric vehicles, told the committee that while the level of fundamental research on battery technology is respectable, it is not matched by a comparable effort in research on motor design, control and body design. Others, however, considered that the balance is about right.

Whether further support is necessary and, if so, whether it should come from government or private forces will no doubt figure in the final report. The Department of Industry, which has put up most of the government money, points out that in allocating its resource it has to balance the potential advantages of spending more on electric vehicles research with spending more on improving the efficiency of the internal combustion engine. It is currently reviewing its priorities.

Judy Redfearn

Thalassaemia

Saudi-London plan

Saudi Arabia has signed a contract worth £1 million over 3 years for joint research on thalassaemia — a crippling genetic blood disease affecting malaria-belt countries. The contract is between two London medical schools — University College Hospital Department of Obstetrics and St Mary's Hospital Medical School Department of Biochemistry — and King Abdul Aziz University, Jeddah.

Negotiations began in 1976 — when the then Saudi Ambassador in Denmark, Sheikh Faisal al-Hegelan, took his 14-year-old thalassaemic son Khaled for treatment at UCH. Khaled was treated by Dr Bernadette Modell, and his parents wanted to know everything about his treatment. (It involved intensive blood transfusions with nightly injections of desferrioxamine to remove excess iron.)

Dr Modell — who has worked mostly with the Cypriot community in London — explained that support for clinical and fundamental research into the disease was lacking in Britain, where it is an uncommon



condition. But in Saudi Arabia it is quite common and it is likely to emerge as a major medical burden now that adequate pre-natal and post-natal care is allowing thalassaemic babies to survive.

The Ambassador discussed the problem with Prince Sultan — Minister of Defence in Saudi Arabia — who immediately offered \$100,000. He sent it to Khaled's mother in England, with the request "to build on this". He would back similar research in Saudi Arabia, he said.

Prince Sultan asked for the fund to be named after his father, King Abdul Aziz, and this may have encouraged other princes to contribute. Prince Nawaf offered \$30,000; and Prince Salman bin Abdul Aziz gave £10,000 to the Thalassaemia Society, a group of parents and sufferers. The boy Khaled (who is now 18 and has a good prognosis) himself approached Sheikh Yamani, the Saudi oil minister, who chipped in £15,000 for UCH through Aramco. Dr Modell suddenly found her research — which had rubbed along on £800 a month from private patients and one Medical Research Council technician — could develop.

Meanwhile the wife of Sheikh Faisal — the Ambassador — had asked Dr Modell and her collaborators, Professor Denys Fairweather (professor of obstetrics and Gynaecology at UCH) and Professor Bob Williamson (professor of biochemistry at St Mary's preclinical school), to provide a letter explaining their research and its needs. The result was that Crown Prince Fahd encouraged a link between King Abdul Aziz University and the London schools. Saud Sejeny — the Vice Dean of the medical school — thought that research should be a major element in the contract, and so it has proved.

The £1 million will be divided roughly 50:50 between the UK and Jeddah. The British scientists and doctors will go for short visits to Jeddah, and members of the Jeddah team will travel to Britain for training.

At Jeddah departments of haematology, obstetrics, paediatrics and community medicine will be involved. "We have as much to learn from them as they from us" said Dr Modell. Saudi Arabia is determined to adapt Western medicine to a form appropriate to its own culture and community needs. For example, the system of prenatal diagnosis which has been very effective in combatting thalassaemia in the London Cypriot community may not be acceptable for Saudis, as it involves sampling fetal blood at 18 weeks of pregnancy (before that the fetus is too delicate) and abortion at 20 weeks if there is a positive indication. Also there is a high rate of cousin marriage in the country, so that the question of genetic counselling would be complex.

The first step of the contract will be to export methods of clinical treatment, and help standardise techniques for diagnosing haemoglobinopathies. Jeddah already

works in this area but London will provide "that extra tier of research thinking". The second step will be to extend research, and at Jeddah particularly to mount several surveys to help determine the genetics of thalassaemia syndromes — of which there are more than one.

Future hopes lie with developing new methods of treatment, and with earlier prenatal diagnosis (towards which Professor Williamson has had recent success — see *Nature* 285, p144; 1980). All avenues are open in the contract and, says Modell "the Saudis will wait to see how this goes. If it goes well, they may design some other projects".

Robert Walgate

Defence research

New customers

The British Ministry of Defence is on the way to being a substantial backer of university research. The ministry's spending on university research, amounting to £2.75 million in the financial year 1978-79, had increased to £4.2 million in 1979-80 (both figures in 1978 prices). And there is at least some hope of further growth to come.

Much of the credit goes to Professor Ron Mason, Chief Scientist at the ministry for the past two and a half years, but previously a chemist at the University of Sussex. Although his predecessors in the post have usually come from universities, he seems to have been especially keen that more defence research should be done there. Some of the topics the ministry is just now keen to foster through defence seminars for university staff and industry are operational analysis, surveillance techniques, new techniques arising from space research, applied psychology in man, machine intelligence and signal and image processing.

The stagnation of research council budgets has helped the MOD to open university doors. Mason says that researchers have begun to put aside their scruples about accepting defence money now that funds from conventional sources are scarce. But there is a snag — the general demise of the dual-support system has meant that many universities lack the basic facilities which universities used to offer. The universities have also become less attractive to external bodies seeking to place research contracts as they have become less able to offer jobs to bright young people.

Professor Mason says that the universities are now more sluggish in their response to urgent research needs than even two or three years ago. His problem is that the ministry's own research establishments (34 of them) are no better. There, too, most vacant posts go unfilled.

The universities, therefore, have not lost all their appeal. There are two ways in which Professor Mason has tried to tap their resources for defence research. He is

most proud of his defence seminars intended to tell academics what the MOD is looking for and to let Mason and his colleagues know what universities have to offer. So far, there have been five seminars, most of which have led to firm research contracts. Several more are planned.

Mason also hopes for benefits from closer cooperation with the Science Research Council. Both organizations have to tread warily however because of possible conflicting interests. The MOD is oriented towards problem-solving for its own ends whereas the SRC's aim is to support research of 'timeliness and promise'. Nevertheless, says Mason, the SRC has recently become more willing to pick out topics such as polymer science and marine engineering for special attention. He sees no obstacle to future cooperation on promoting areas of mutual concern, systems analysis and space systems for example.

Talks with the SRC chairman on the former and a multi-lateral debate on the latter are already under way. Britain has wound down its national civil space programme to the extent that British scientists now have to rely on the European Space Agency for facilities. Mason, however, reckons that there is a growing interest in space among British industry which could be used to benefit both civilian and military users.

Mason's clout may be enhanced because the defence research budget does not appear to be as much under pressure as the civil research funds. The government recently announced its intention of sustaining a 3 per cent annual growth in real terms in defence until 1986. But there are problems, for defence research, involved as it is with high technology, tends to increase in cost more rapidly than inflation. Next year, Britain will spend 13 per cent of its defence budget on research and development, £1,600 million out of £10,800 million. But is the balance right, and how much should be spent on long-term as opposed to short and medium-term research? Unfortunately, says Mason, there is no way of estimating a correct balance.

The MOD research establishments have been mainly concerned with short and medium-term research, and Professor Mason believes there is a need to increase their innovative function. The MOD has recently reviewed the role of the defence research establishments so as to assess how much of their responsibility could be transferred to industry and the universities. That review is due to be brought up in Parliament before the summer recess.

In the meantime, Professor Mason says there is a need for more collaboration on research between government agencies. "The government enforces a policy of water-tight compartments. But we can't afford, given the resources, an isolated policy", he says.

Judy Redfearn

French research

Better budgets

THE French government has proposed a 20 per cent increase in spending on research and development in 1981, as part of its plan that research and development should increase to 2.3 per cent of gross domestic product by 1985. The plan is part of the French budget and remains only to be approved by the Assembly and Senate. A total of 410 jobs would also be created, mostly at the senior postdoctoral level of 'chargés de recherche'.

French science spending, as a proportion of GDP, fell rapidly behind that of other countries in Europe during the 1970s. At present, France spends 1.8 per cent of its GDP on research and development, counting government-backed and industrial science together, compared with nearer 2.2 per cent in other large Western countries. According to figures published by the European Commission, the ratio of growth rates of government science spending and GDP in France between 1970 and 1977 was 0.79, the lowest in Europe. The highest was Ireland with 1.35; then came West Germany (1.25), The Netherlands (1.11), Denmark (1.9), the UK (1.01), Italy (0.94) and Belgium (0.92).

For this reason, Pierre Aigrain, Secretary of State for Science in the French government, has had little difficulty in convincing other ministers that the next five-year plan (1980-85) must show a substantial increase in science spending by government.

However, the recommendations of a controversial report — the Faroux report — on industrial research and development have not yet found favour. Roger Faroux, director-general of the chemical firm Saint-Gobain-Pont-à-Mousson, and his group reported to Aigrain in March that research spending in industry should increase by 65 per cent over the next five years, and that tax incentives should be used this end. The 65 per cent increase compares with the 40 per cent 'in a few years' set as the target by the Council of Ministers in August 1979; thus Faroux is recommending a substantial increase of the proportion (at present 42 per cent) of industrial research in the total research budget.

One of the principal beneficiaries of the new proposals will be the Centre National de la Recherche Scientifique (CNRS), which accounts for a large part of France's basic research. Its budget will rise 20 per cent from £874 million in 1980 to £1,040 million in 1981. (These sums include the salaries of some 20,000 scientists and staff.) Within CNRS, the biggest increase will come in 'moyens direct' — money which will be paid to laboratories without the need for approval by subject committees, and which will be used to buy and replace small to medium items of equipment (infra-red spectrometers, for example).

The CNRS has also been pressed to increase its connections with industry, and to this end has appointed a 'monsieur rayonnement' who will attempt to place departing senior CNRS scientists in industrial jobs — and also in ministries, where CNRS feels it should be better understood. There is now discussion in France as to whether to set up small scientific advisory groups in the ministries, rather on the lines of the 'chief scientist' departments in the UK; this talk is the consequence of the division of Aigrain's empire earlier this year between the Minister of Industry, M. Giraud, and himself. Giraud has taken on responsibility for the space, nuclear and other large projects that were once Aigrain's concern.

At CNRS, it has not been decided how to allocate the 240 new posts which it will receive under the new proposals; but, said a spokeswoman, 'the life sciences will be well off'. Of the other posts created by the government, 55 would go to INSERM (Institut National de la Santé et de la Recherche Médicale) and 38 to INRA (Institut National de la Recherche Agronomique).

Robert Walgate

More cosmonauts

French this time

FRANCO-SOVIET space cooperation took another step forward last week when the French National Centre for Space Studies (CNES) announced the selection of two air force fighter pilots to train for a Soyuz-Salyut orbital link-up scheduled for mid-1982. The two 'spationautes', Lieutenant-Colonel Jean-Loup Chrétien, aged 40 and Commander Patrick Baudry, aged 34, will travel to the Soviet cosmonaut training grounds 40 kilometers outside Moscow on September 1 where they will undergo a year and a half of training with Soviet colleagues. The mission calls for a Soviet pilot and a French co-pilot, who will be selected from one of the two French candidates, to spend a week on board the Salyut orbital station where they will conduct a series of eight experiments. Four of the experiments are in the area of the physiology and biology of space including an investigation of the control of bacterial infections in space environments and the effects of weightlessness on the flow of blood in the human body, two are in material science with special reference to the creation of aluminium-indium alloys and two will investigate high resolution space photography. The cost of the programme is 30 million FF.

The Franco-Soviet project is seen in France to come at a politically favourable time following the recent diplomatic meetings in Warsaw and the decision of France to participate in the Moscow Olympics. But Franco-Soviet space cooperation dates to a 1966 agreement negotiated by General de Gaulle for the

purpose of "special cooperation in space research". Since then France has launched a series of satellites on Soviet rockets including the 1977 Sign-3 gamma-ray astronomy satellite and a series of three SRET satellites between 1972 and 1975 to explore cooling and radiation protection problems in space technology. Since 1977, the fields of the biology of weightlessness and space crystallography have been opened up with French and Soviet scientists collaborating on the data collected by French satellites. Future joint experiments envisaged are explorations of the planet Venus including a balloon experiment to investigate the Venusian atmosphere planned for 1984. But the main thrust of French space research, which included collaboration with Germany and the US as well as the USSR, is in applied space research.

Joe Schwartz

Baltic oil

Moving offshore

Next month, Petrobaltyk, the consortium of Soviet, Polish and East German oil interests, will sink its first offshore bore-hole in the Baltic. This move follows four years of intensive prospecting, which, according to T. G. Vekilov, Deputy Minister of the Soviet Gas Industry, will continue in parallel with the drilling operations. Vekilov was also careful to point out, in a *Pravda* current affairs interview, that there is positively no energy crisis facing the Soviet Union, whose fossil fuel reserves are among the largest in the world. Soviet interest in possible oil or gas beneath the Baltic is, Vekilov implied, simply another aspect of the "forward thinking about future energy" urged by Mr Brezhnev at the 1979 Party Plenum.

For Petrobaltyk, this forward thinking began in November, 1975, when the three participating states signed an agreement about joint prospecting for oil and gas on the Baltic shelf. The area was, on the face of things, promising. Estonia has extensive oil shale deposits, and some small oil and gas deposits had been located in northern Poland.

The primary task of Petrobaltyk, to date, has been to carry out a major seismic survey of the Baltic — one interesting spin-off of which has been the development of a prospecting method based on anomalies of the natural electric field. By 1978, a team from Leningrad had established a set of tectonic, lithologic and hydrogeological indices for oil-bearing formations in the palaeozoic formations of the peribaltic syncline, and had deduced that for the Cambrian and Devonian strata, prospects increased from east to west, while for the Ordovician and Silurian strata the opposite is true.

Owing to the multinational structure of Petrobaltyk, however, publication of the results of individual surveys has been restricted and geologists taking part in the

surveys have been even more than usually reticent about their preliminary findings. For a time, plans were afoot to buy or hire a Vexco drilling rig, but when these had to be abandoned for lack of hard currency, the shut-down of information was total.

Petrobaltik's own self-produced rig, capable of operating in up to 90m of water, is now moored at Gransk, waiting to go into operation. A few weeks ago, the Helsinki Convention on the Protection of the Marine Environment of the Baltic Sea Area finally became law, binding on all states of the Baltic littoral. A major point of the convention is the prohibition of oil dumping and an appeal to the contracting parties to take measures to prevent pollution resulting from the exploration and exploitation of the sea-bed and its subsoil.

Rather surprisingly, although the Soviet media paid special attention to the Baltic republics and sea in their routine annual coverage of "environment day" (June 5), the emphasis was on effluent dumping, the need for on-shore purification installations, ecology courses in universities, and the necessity for international ecology film festivals.

No mention was made of Petrobaltik, or of what measures it proposes to take in the case of oil leakage. This may, of course, simply reflect well-placed confidence on the part of the planners. The Finns of the Åsland Islands, who caught the main brunt of an oil-slick from the Gulf of Riga last year, have however expressed considerable concern; while on the south-east Baltic coast-line, the citizens of the contracting states of Petrobaltik, though less vocal, are known to feel a similar concern.

Vera Rich

London Zoo

More falling-out

THE London Zoo has run into another spot of bother. Earlier this week, it became known that Mr Michael Hanson, the newly appointed director of administration at the Zoological Society of London, had resigned his post and would be leaving on 14 August.

Mr Hanson, whose responsibilities include the management of the establishment and the finances of the Zoo, was recruited from the British Civil Service in September 1979. It has been apparent for some time that the chief task of the administrative director is somehow to bring back to balance the Zoo's trading account, either by making sure that enough small children ride enough elephants at a sufficient price to keep all the animals (and their keepers) fed and housed or by some other means.

There is every reason to accept the statement put out by the Zoo earlier this week that Mr Hanson's resignation is unconnected with that of Dr Ronald



Zuckerman is the Zoo

Hedley, the part-time Secretary of the Zoo. Hedley, now Director of the British Museum (Natural History) is said to have been murmuring about overwork for several months.

The joint departure may however provide a chance for making a substantial appointment. The top establishment includes three directors (the other two responsible for animals and research), a secretary and a president, now Lord Zuckerman. Lord Zuckerman, by temperament and inclination, functions very much as an executive president — one council member said this week that 'Solly is the Zoo'.

Concern that he could not get on with the job for which he was hired without detailed scrutiny from above appears to have been one cause of Mr Hanson's departure. Recent meetings of the council have also dwelt on Mr Hanson's plans for re-organisation, considered to have been imaginative and yet similar in their essentials to plans out forward earlier.

When Mr Hanson first tendered his resignation, he was apparently asked by the council to reconsider it, but declined to do so. It is not known what may have passed between him and Lord Zuckerman.

Patent law

Bugs protected

BY the narrowest of margins, the US Supreme Court had decided that living micro-organisms can be patented. In a verdict announced on Monday, the court ruled by five to four that there is nothing inherent in existing US patent law which prevents an invention from being patented just because it is alive. The court had been asked to decide on an appeal by the Patent and Trademarks Office against the decision of a lower court to grant a patent to Dr. Ananda M. Chakrabarty of the

General Electric Company for an artificially bred strain of the bacterium *Pseudomonas*, first developed to help clear up oil spills by degrading different chemical components.

Dr Chakrabarty's bacterium was not created with the use of recombinant DNA techniques. However it does fall within the general category of genetic engineering — and the court's verdict is seen as a welcome boost by pharmaceutical and other biotechnology companies which, together with several US university research groups, have had patent decisions on new micro-organisms held up until the Supreme Court's verdict was known.

The patent application had been rejected twice by the Patent Office, largely on the grounds that, in writing the original patent laws in the eighteenth century and in discussing their subsequent revisions, Congress had at no time indicated that living organisms were explicitly included.

Wide-ranging arguments had been brought in by both sides. Those supporting the patent application argued that it should be granted because of the economic importance of micro-organisms as part of the explosion of interest in biotechnology; opponents claimed that it would legitimize interference with natural processes in general, and should also be treated with particular care because of the potential health hazards.

In the end, however, the court's ruling was based on a narrow interpretation of congressional intent in writing the patent laws. Chief Justice Warren Burger, with four of his colleagues, argued that just because living matter was not mentioned in the patent laws, this did not mean it was excluded; the minority, led by Justice Brennan, argued conversely that, because of this very ambiguity, the issue of patentability should be decided by Congress rather than the courts.

Central to the case were the implications of a special law passed by Congress in 1930 allowing for the patenting of asexually reproduced plants, with an additional act in 1970 extending protection to new plant varieties capable of sexual reproduction.

The US Patent Office had argued that, unless Congress had intended the original act to exclude living matter, this additional legislation should not have been necessary. The minority agreed.

Chief Justice Burger, however, invoked the argument of Thomas Jefferson — the original author of US patent legislation — that "ingenuity should receive a liberal encouragement" to confirm that a novel micro-organism can legitimately be considered as a 'manufacture' or 'composition of matter' under the terms of the Patent Act of 1793.

The Supreme Court's decision, which finally ends almost eight years of legal debate, brings the US in line with several European countries, such as the UK, which already allow living organisms to be patented.

David Dickson

NEWS AND VIEWS

Eruption of Mt. St. Helens:

Seismology

from the Geophysics Program*, University of Washington

ON May 18, 1980 at 0832 local time a major geological event occurred with the cataclysmic eruption of Mt. St. Helens in Washington's Cascade Range. The eruption followed two months of intense seismic activity, surface deformation, and sporadic, but minor steam and ash eruptions. These geophysical and geological precursors signaled the reawakening of the volcano after a period of quiescence since activity was last recorded in 1856 (Crandell & Mullineaux *Science* **187**, 438; 1975; Crandell & Mullineaux, *US Geol. Survey Bull.* **1383-c**, 25p; 1978).

The cataclysmic explosions of May 18 caused an estimated \$2 billion or more in damage, although the volcano lies in a relatively remote area. The cost in human life has been substantial with 22 confirmed dead and 60 to 70 still missing at the time of writing. A large sector of the north and northeast side of the mountain was devastated by pyroclastic flows, mudflows and tephra fallout. The direct blast from the explosions leveled entire forested areas on the north side; the affected area is estimated to be 400 km². Two river systems with sources near the mountain have been extensively altered by mudflows and flooding. The morphology of the volcano has drastically changed from a nearly symmetrical cone to an asymmetric edifice approximately 400 m lower in height, although much of the south and west sides of the volcano remain relatively untouched by the explosion.

Seismic activity was the first indication of the reawakening of Mt. St. Helens, beginning abruptly with an earthquake of magnitude 4 at 1547 local time on March 20, 1980. This earthquake was located just north of the summit by the regional

seismograph network which included one station 3.5 km west of the summit. Since this was the largest earthquake recorded in the southern Washington Cascade range during seven years of instrumental observations, an aftershock study was begun on March 21 in an effort to examine seismo-tectonic processes near Mt. St. Helens. To improve the seismic coverage in the area, three portable seismic recorders were installed within 15 km of the summit, and a second telemetered station was added to the permanent network 35 km northeast of the epicenter.

A rapid increase in the number of small shocks made it clear that an unusual earthquake sequence was beginning at Mt. St. Helens. The initial aftershock activity failed to follow the usual decay in the number of events with time, and on March 22 a second magnitude 4 earthquake occurred in the same region. By March 24, additional earthquakes larger than magnitude 4 had occurred and the general seismicity increased to the point that a volcanic mechanism was required to

explain the concentrated, high rates of seismicity. Additional temporary seismograph stations were installed to provide data adequate for obtaining accurate hypocenter locations in the hope that careful tracking of the hypocenters would increase our overall ability to monitor the volcanic hazard. Twenty four hour observation of the seismographic records began on March 24, and this effort has continued to the present as part of the hazard warning effort. A final, dramatic increase of seismic activity occurred on March 25, when the rate of seismicity reached its peak. Seismic stations within 8 km of the summit were continuously saturated, and individual earthquakes could no longer be distinguished from background seismic levels. More distant stations were used to resolve individual earthquakes for determining occurrence rates, magnitudes, and other earthquake parameters.

On March 27, two days after the seismicity peak, the first steam eruption occurred at 1236 local time. Moderate steam and ash eruptions continued for the next few weeks, declining in frequency until by April 23 only occasional small steam bursts were observed. On May 8 steam and ash eruptions resumed, occurring periodically for several days. Between May 14 and the cataclysmic eruption on May 18 there was only minor eruptive activity.

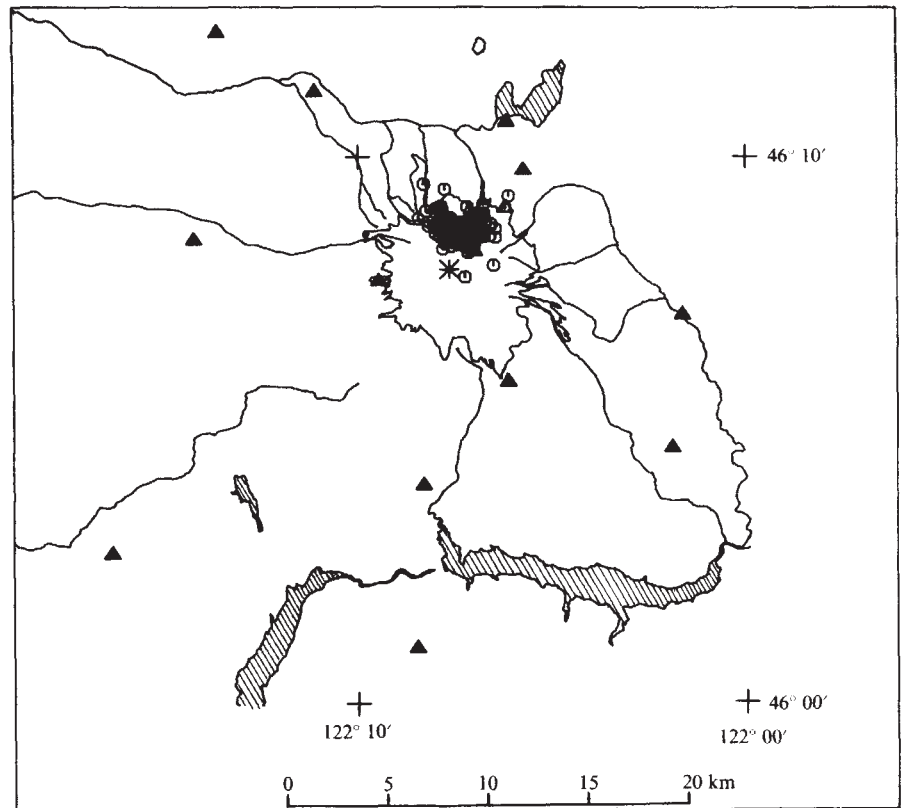
As the seismic energy release beneath St. Helens occurred at a high rate, count statistics for the whole episode have been kept only for earthquakes exceeding magnitude 3.2. After reaching a peak of 8 to 10 earthquakes per hour at this magnitude threshold during the evening of

**The seismic monitoring of Mt. St. Helens has involved a large number of individuals, many of them students and staff of the University of Washington Geophysics Program. This preliminary, descriptive report was compiled by the following people, listed alphabetically: R.S. Crosson, E.T. Endo, S.D. Malone, L.J. Nosen, and C.S. Weaver. Crosson is a Professor of Geophysics, Malone is a Senior Associate, and Nosen is a network seismologist, all with the University of Washington. Endo and Weaver are geophysicists with the US Geological Survey, Menlo Park, California, and both are currently on assignment in Seattle. Significant contributions to the seismic monitoring were made by J.M. Coakley and E.E. Criley (both USGS) and by J.W. Ramey and E.H. Wildermuth (both UW).*

March 25, the rate of activity declined irregularly until the explosive event of May 18. Figure 1 shows a smoothed curve of the rate of occurrence against time. The rate of seismic energy release generally follows the count curve although the decrease is not as great. This reflects the fact that large earthquakes continued at a slightly increasing rate during April and May, while the smaller events declined in frequency. Earthquakes larger than magnitude 4 occurred at an average rate of 5 per day in early April and 8 per day during the week preceding May 18, while the number of events larger than magnitude 3 went from 77 per day to 28 per day during the same period. The largest earthquakes recorded were approximately magnitude 5 and occurred late in the sequence. We estimate the total seismic energy release to date to be equivalent to a single magnitude 6.7 earthquake.

Several periods of harmonic tremor were observed. Normally, a nearly monochromatic 1 Hz signal, lasting from a few minutes to half an hour, was observed on stations within 30 km of the mountain. In several cases these signals were large enough to be observed on seismic stations 250 km distant. There was no apparent correlation of these monochromatic tremor periods and eruptions or unusual earthquake activity. During the intense earthquake activity of March 25-26 harmonic tremor would have been completely masked by the earthquake signals.

By May 1, a total of 15 seismograph stations were operating within a radius of 32 km of the summit. The station distribution is excellent for control of hypocenter coordinates though the velocity model is still poorly known for the immediate area. Virtually all of the earthquakes occurred in an area of 5 km radius centered approximately 2 km directly north of the summit crater (Figure 2). Depths ranged from 0 to about 5 km with a few events possibly as deep as 10 km beneath the average topographic surface.



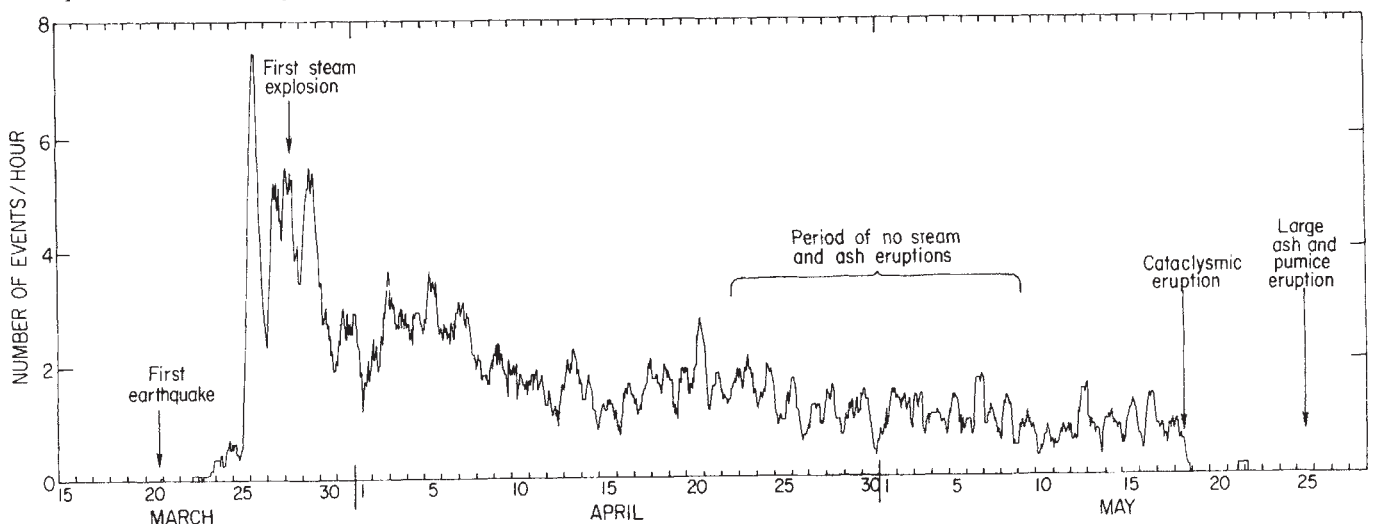
Map of epicenters above approximately 3.2 for period May 1, 1980 to May 18, 1980. Epicenters are open circles, seismograph stations are solid triangles, and bodies of water are shaded. Tree-lines and rivers are drawn. Dark area is epicenter cluster centered 2 km north of the summit.

At this preliminary stage of investigation, we have not been able to obtain good depth measurements of very shallow earthquakes, but it seems probable that many moderate earthquakes (magnitudes 3 to 4) occurred at shallow depths near the base of the volcanic edifice or up in the cone itself.

The blast of May 18 was not preceded by any anomalous seismic activity on a time scale of hours to days. At 0832 local time, an earthquake occurred at a depth of 3 km beneath the volcano. This event may have triggered a landslide off the north side of the mountain which led immediately to the explosion. The details of the explosion and

its relation to the earthquake and landslide have not yet been worked out. A standard Wood-Anderson seismograph in Seattle recorded a magnitude 5.1 event at this time though the signals are more complicated than expected for a single earthquake. After the initial seismic event was over (it lasted for over 8 minutes) the earthquake activity dropped back to a level of only one or two discrete events per minute. This period of relative quiescence lasted for over three hours when both the earthquake activity and volcanic tremor increased. There was a steady increase in the level of seismic activity from 1140 PDT until 1530

Earthquake occurrence rate against time for the Mt. St. Helens eruptive sequence.



PDT when all seismic stations within 100 km were completely saturated and strong tremor was recorded 250 km away. Around 1730 PDT the tremor and earthquake activity abruptly diminished.

Since May 18 the earthquake activity dramatically declined until by the end of May there were only a few small earthquakes per day in the vicinity of the mountain. There was a moderate earthquake swarm coincident with an ash eruption on May 25. Low amplitude seismic noise, often with tremor-like characteristics, has been recorded nearly continuously since the May 18 eruption; although the noise is usually only monitored on the two stations still operating on the flanks of the mountain.

Comparison of the St. Helens sequence with the eruptive behaviour of other volcanoes may yield clues to the physical processes involved and to the predictable aspects of these processes. A particularly interesting example is the eruption of the Kamchatka volcano Bezymianny in 1956 (Gorshkov *Bull. Volcan.* 20, 77; 1959). This volcano went through a cycle remarkably similar to that of Mt. St. Helens: a) a period of almost one month of volcanic earthquakes, b) strong ash eruptions lasting over one month, c) a stage of moderately declining activity lasting nearly 3 months, d) a gigantic explosion approximately six months after initial activity, and e) a post-eruptive stage of about six months of declining but sporadic activity. Except for the time table which is longer and the fact that earthquakes were estimated to be of greater depth (up to 50 km) in the case of Bezymianny, these two volcanic histories are sufficiently similar to suggest the possibility that a moderately predictable process is involved. The explosion of Bezymianny was in fact accompanied by a strong earthquake although the exact time sequence is not as well known as at Mt. St. Helens. The resultant craters of the two volcanoes are also very similar in size and shape.

Our preliminary conclusions are that seismicity provides an intermediate term warning of explosive volcanic hazard (scale of weeks to months) but no apparent short term warning (hours to days). The rate processes which may be extracted from the seismic data such as the strain energy release rate may be valuable in establishing the overall magma injection characteristics and other variables in the volcanic cycle when a sufficiently good understanding of the physical processes is available. Other measurements such as ground deformation must be made to provide basic information upon which to model the entire process. Unfortunately, it is still not clear that reliable short term prediction is feasible. Considering the wealth of seismic data that we have obtained, along with the variety of other observations made on Mt. St. Helens, a unique opportunity exists to probe the inner workings of an explosive volcano. □

Volcanology

from Robert L. Christiansen*

THE explosive eruption of Mount St. Helens completely destroyed its north flank, opening a crater 1.5 km wide, and producing an eruption cloud that deposited a blanket of ash over a large area of the northwestern United States. The eruption, the culmination of a series of seismic and eruptive events that began in late March, was notable for the rapidity with which activity began and progressed and for the magnitude of energy released, both seismically and eruptively. Although further eruptions (including the eruption of lava into the volcano's crater) are likely, it seems probable that the greatest energy release occurred within two months of the initial earthquake of the sequence.

Activity began with a single shock of magnitude 4 on March 20 and grew within 5 days to a swarm in which magnitude 4+ earthquakes occurred at a rate of more than 8 per hour. An interesting decrease in seismicity (although to rates including more than 5 earthquakes of magnitudes 4+ per day — high by any ordinary standard) occurred during the day and a

half before the first eruption, which was a crater-forming burst beginning at 1238 on March 27.

After a pause until about 0300 the following morning, the volcano erupted again, this time for a sustained period of nearly two hours. Similar eruptions continued for the following four days, with both short, essentially single-burst eruptions and sustained longer eruptions. The eruptions were all probably steam-generated and produced only lithic-crystal ash that apparently was derived by shallow explosions within a 350-year old summit dome. A moderate amount of this ash was distributed 50 km away and some was reported as far as 100 km to the east, but most of it fell within 5-20 km of the volcano's summit.

Phreatic eruptions continued after April 1 but were mainly of short duration and occurred at successively longer intervals. They ceased temporarily after a small eruption that produced ash on the volcano's upper flank on the morning of April 22. Up to that time the summit crater,

The new crater formed by the explosive eruption.



from which all of the eruptions had occurred, was nearly free of steam between eruptions except for areas of diffusely steaming ground. By contrast, during the late April to early May pause in eruptions, fumaroles vented continuously from several points within the crater. Weak to moderate phreatic eruptions occurred again for a few days in early May but once again ceased while fumarolic steaming continued in the crater.

The typical spring weather of Mount St. Helens made observational conditions poor for most of the time. The mountain was not visible by air from late morning of March 25 until a few hours after the eruption began on March 27. When the view cleared that afternoon, a remarkable series of changes was apparent at the volcano's summit and on its upper north flank. During the period of peak seismicity and initial eruption, a nearly continuous east-trending fracture system 5,000 m long had formed across the summit, nearly bisecting the old snow-and-ice filled summit crater. Another somewhat less continuous system of fractures paralleled the major one along the old north crater rim and bounded a block of the volcano's north flank that was conspicuously displaced above the former elevations of the flank. Later photogrammetric measurements showed this block of the north flank to stand more than 100 m higher than the corresponding previous points on the flank; much of the apparent discrepancy in elevation was, however, due to northward displacement of the flank rather than true uplift.

Deformation of the north flank continued after the initial displacement, forming a conspicuously visible bulge of both the glaciers and the rock edifice of the volcano. Continued photogrammetric re-contouring of this north flank was coupled with frequently repeated geodetic measurements and tilt monitoring to study a remarkable rate of deformation on the north flank. A total northward displacement of over 100 m occurred during the nearly two months from the time of initial formation of this bulge until the climactic eruption of May 18. The rate of deformation at each surveyed point remained quite constant throughout the period of intensive observation, which lasted from April 25 until the morning of the major eruption. Rates in the fastest-growing parts of the bulge were 1.5 to 2 m per day; the displacement vectors were nearly horizontal toward the north-northwest.

Although it was hoped that changes in seismicity and in the rate of deformation on the north flank, as well as other precursors, might give warning of a major slope failure, the possibility that such a failure might occur resulted in the closing of the area north of the volcano by civil authorities. That such a slope failure might trigger an eruption was also considered a distinct possibility. The event finally

occurred, however, essentially without warning at 0832 on the morning of May 18. It appears to have been triggered by a large earthquake, which probably had a Richter magnitude of 5 (unusual wave characteristics preclude a simple evaluation of the magnitude of the event). The earthquake shook the walls of the summit crater, causing numerous avalanches, and the entire north slope of the volcano began to separate from the main edifice along a crack that opened across the upper part of the bulge. A small dark ash-rich eruption cloud emerged from this crack as the slope failed in a catastrophic avalanche below.

Within seconds, the avalanche was overtaken by a large laterally directed blast (see cover pictures) that carried lithic ash and lapilli in a devastating hurricane over a northward sector of nearly 180 degrees, 30 km across from west to east and extending outward more than 20 km from the volcano's summit. In an inner zone of nearly 10 km width virtually everything in the path of this blast was destroyed, and no trees remain in that formerly densely forested area. Beyond, to nearly the limit of the blast, all the trees were blown down to the ground, and at the blast's outer limit the trees were thoroughly seared.

The avalanching north flank raced down the slope of the volcano, slammed into a ridge about 8 km to the north, displaced the water of Spirit Lake, raising its bed by more than 60 m, and formed a hummocky debris flow that locally crossed the ridge to the north but mainly turned and flowed down the valley of the North Toutle river for 18 km. The displaced water of Spirit Lake, melting blocks of ice from the former glaciers of the volcano's north flank, water from the displaced river bed, and melting snow and ice on the volcano's remaining slopes produced mudflows that flooded the debris flow and generated floods all the way down the Toutle River, the Cowlitz river below it, and into the Columbia River.

The initial events of the eruption — the avalanche-debris flow and the lateral blast — caused most of the casualties and destruction in the region of the volcano. However, before the lateral blast had reached its full proportions a vertical eruption column began to rise from the position of the former summit crater and

within less than 10 minutes had risen to a height of more than 20 km. Ash from this eruption cloud was rapidly blown northeastward, producing lightning and starting hundreds of small forest fires, causing darkness as far east as 100 km, and depositing lithic-vitric ash for many hundreds of kilometers. Major ash falls occurred as far east as central Montana, and ash fell visibly even in the Great Plains of the central United States. As this plinian eruption column formed, it reamed out the volcanic conduit, forming a central crater more than 1.5 km in diameter. This crater, along with the north flank that was entirely removed by the initial avalanche and blast formed a great amphitheater 1.5 × 3 km across, enclosed by the volcano's former east, south, and west flanks.

As the plinian column continued to erupt vigorously for more than 9 hours before gradually decreasing in height and intensity, it produced numerous ash flows. The first of these were thin flows that spread out over the entire upper surface of the volcano and were generated by both the initial blast cloud as it expanded in the vicinity of the summit and by the plinian eruption column. Later, the ash flows were all directed out through the large northward breach of the crater to form a fan of pumiceous ash flows over the debris flows, extending past Spirit Lake and part way down the former drainage of the Toutle River. These ash flows continued to be observed until dark on the 18th. The hot blast deposits, the debris flow, and these ash flows were frequently disrupted in the vicinity of Spirit Lake and the former Toutle river by large phreatic eruptions that blew through them to form craters as large as 20 m across and drove columns of ash to heights as great as 2000 m above the surface (see picture opposite).

The magma produced during and since the major eruption of May 18 has been dacite. The principal juvenile material is light-colored hypersthene-hornblende dacite having a range of silica content from 64% to 68% (chemical analyses by K. Wozniak, S. Hughes, E. M. Taylor of Oregon State University). In addition, there is a darker, denser dacite whose composition has not yet been determined but that appears also to be juvenile. The eruption had already begun to decrease in intensity by about 1700 on Sunday May 18th, but continued intermittently for the rest of the week. On the following Saturday night a series of increasingly energetic ash eruptions led to the second largest eruptive event on Sunday May 25. That eruption began at about 0230 during a period when winds were erratic, having greatly different directions at different elevations. Although the eruption was an order of magnitude less voluminous than that of the 18th, it scattered ash over wide areas of western Washington and Oregon.

Since the end of the eruption of May 25, late in the day, the volcano has continued to emit large quantities of steam that

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One of them Dave Johnston, a geologist with the USGS, was killed in the cataclysmic blast that began the May 18 eruption while manning an observation post 8 km north-northwest of the mountain. Dave had been involved in the monitoring of Mt. St. Helens from the beginning of the seismic sequence, and had hoped to be able to issue a short term warning of the impending eruption.



Spirit Lake on May 29, 1980

condense in the air to form plumes that rise to altitudes of 3 to 5 km above sea level. The odor of sulfur gases is prominent in these plumes, but relatively little ash has been carried by them, and almost none has fallen more than a few kilometers beyond the volcano.

No lava has yet appeared at the surface. There have, however, been several night observations of incandescent rock, probably caused by hot gases streaming through the vents from a magma body not far below. The rapid pace and high rates of energy release to date, from the initial earthquakes, through the early phreatic

eruption phases, to the explosive magmatic eruptions of the last few weeks are noteworthy. That magma will yet reach the surface to form a volcanic dome, whose emplacement would possibly be accompanied by further explosive eruptions, seems distinctly probable. Further disruption and rebuilding of such a dome could continue for weeks, months, or years to come, but it seems likely that the volcanic edifice will be rebuilt by the outpouring of both dacitic and andesitic lavas, as has happened so many times before in the brief but eventful history of Mount St. Helens. □

Effects on climate

from C.B. Sear and P.M. Kelly

Now that the dust has settled over the United States after the explosive eruptions of Mount St. Helens, attention will turn to the longer term effects of these events on world weather and climate. There has been much concern in recent years over the possibility that anthropogenic increases in atmospheric carbon dioxide will produce a major climatic change over the next fifty to one hundred years. Natural mechanisms, such as the level of dust thrown into the stratosphere by explosive volcanic activity, have, however, been held responsible for significant climatic fluctuations in the past, and must also be considered in any attempt to predict the world's future climate. Benjamin Franklin, whilst sojourning in Paris, proposed that the severe European winter of 1783-84 was caused by the eruptions of Icelandic volcanoes in May and June of 1783.

Volcanic aerosols injected through the tropopause into the stratosphere can

remain there for several years. After a few months, under the influence of the stratospheric wind systems, they form a veil which can cover most of a hemisphere and occasionally, after very large eruptions, the whole globe. The spread of a dust veil is dependent on the latitude of injection, the height reached by the dust, the size of the dust particles and the state of the stratospheric circulation during the development of the veil. In general, this circulation tends to restrict veils resulting from northern hemisphere middle and high latitude eruptions to regions north of 30°N, whereas dust from low latitude eruptions, such as those of Krakatau (1883) and Agung (Bali, 1963), tends to spread over the whole globe. In the case of Mount St. Helens, there is a good chance that the dust, although injected into middle latitudes, will spread over a greater latitudinal range than usual. This is because May is the month with the greatest

equatorward component of flow in the lower stratosphere.

The amount of solar energy reaching the Earth's surface is reduced by a dust veil, as the radiation balance is altered by back scattering, forward scattering and absorption within the dust veil. The relative importance of these processes varies considerably with the height of the veil and the size and chemical composition of the dust particles and aerosols. Indeed, very often, several distinct layers occur and calculation of the net effect on the radiation balance is complex. The reduction in the direct solar radiation at the surface is offset to some extent by an increase in diffuse radiation.

In the case of large explosive eruptions, these alterations in the energy budget are believed to be sufficient to produce changes in the atmospheric circulation and climate. Absorption within the dust veil results in stratospheric warming and the reduction in energy received at the Earth's surface leads to cooling in the lower atmosphere. Lamb, in an exhaustive work (*Phil. Trans. Roy. Soc., A*, **266**, 425; 1970), has discussed the effects of these dust veils and has produced a chronology of major explosive volcanic events for the period since AD 1500.

Recently, theoretical modelling has been undertaken in order to provide estimates of the magnitude of the effects of major eruptions on the temperature and circulation of the lower atmosphere. Hunt, (*Mon. Wea. Rev.* **105**, 3, 247; 1977) has estimated the climatic effects of a dust input of Krakatau magnitude using a nine level general circulation model. The maximum depression of hemispheric mean surface temperature was around 0.3°C, occurring about three months after the initial input. Pollack *et al* (*J. geophys. Res.* **81**, 1071; 1976) modelled surface temperature changes during periods of high volcanic activity, such as occurred during the 1880s. They found a resultant cooling of the order of 1°C and stressed the importance of the contribution of sulphuric acid aerosols to the energy balance changes in the stratosphere. On the other hand, Deirmendjian (*Adv. Geophys* **16**, 267, 1973) has concluded that there is little solid evidence for climatological effects of major eruptions in the conventional meteorological parameters.

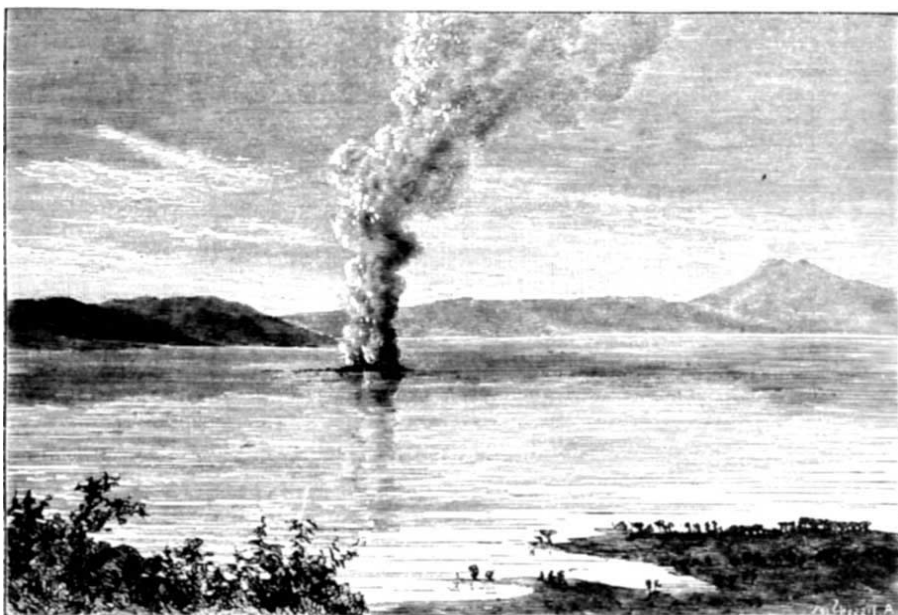
A number of empirical and statistical studies have revealed evidence of volcanic effects on climate and the atmospheric circulation. Mass and Schneider (*J. Atmos. Sci.* **34**, 1995; 1977) have found a short term volcanic signal in air temperature records. Kelly (*Nature* **268**, 616; 1977) has noted the correspondence between long term trends in central England temperature, the North Atlantic westerly winds and the degree of explosive volcanic activity. Bryson and Goodman (*Science* **207**, 1041; 1980) have recently suggested that climate prediction for more than two years ahead will require forecasts



100 years ago

A LACUSTRINE VOLCANO

In a recent number of *La Nature* further details, furnished by the French Consul of San Salvador, M. J. Laferrière, are given concerning the recent volcanic phenomenon in Lake Ilopango in that State. The accompanying illustration, from a photograph, will show the nature of the crater which has risen in the midst of the lake. Earthquakes were felt in San Salvador in the first half of January of this year; there were three strong shocks, less violent, however, than those of 1876. These earthquakes had their centre in the vicinity of Lake Ilopango, in the midst of which rose three volcanic openings connected with each other. This new crater, which, seen from a distance as in the illustration, appears a small islet, rises above the surface of the water, however, about twenty metres. An attempt was made to approach it in a boat, but the waters were all in a state of ebullition from contact with the burning rock, and gave off torrents of steam. An abundant column of smoke rose in the air, assuming the aspect of an immense cloud, which was seen from a great distance, and formed an imposing spectacle. The phenomenon was preceded by an exceptional rising of the lake, increased by the abundant winter rains. According to an old tradition the Spaniards maintain that when the lake rises earthquakes are to be feared. Formerly, also, it was the custom to dig trenches to facilitate the escape of the



Aspect of the Volcano in Lake Ilopango

waters. This practice was followed without intermission for a century, and volcanic phenomena did not appear during all that time. The present phenomena seem to justify this tradition.

If it is difficult to explain the fact it is still interesting to remember that a great number of volcanoes are submarine, that others are found for the most part in islands or in maritime regions, and that water may be one of the feeders of volcanic fires. Lake Ilopango, also known as Lake Conjutepec, is, according to M. Laferrière, a sunk crater. It is in the volcanic line, and it is a general fact in Central America that lakes alternate with volcanic cones. The water of this lake is

brackish, very bitter, and almost viscous. It gives off sometimes, here and there, bubbles of sulphohydric acid gas. The lake is about 12 kilometres long by 16 broad; the depth is unknown. It is about 12 kilometres from the city of San Salvador. The Consul of France in Guatemala, M. de Thiersant, states that Lake Ilopango has now a temperature of 38°C on its shore, and is in complete ebullition round the volcano. All the fishes are cooked and float upon the surface, with a great number of shellfish and other aquatic animals. The volcano continues to rise, and the level of the lake is being gradually lowered.

From *Nature* 22, 10 June, 129, 1880.

of the general level of global volcanic activity. It has been suggested that the high frequency of explosive eruptions during the 17th century and early 19th century may have been a major cause of the most severe phases of the Little Ice Age and that the general warming of the early 20th century was a result of the reduced level of volcanic activity at that time. Miles and Gildersleeves (*Nature* 271, 735; 1978) do not, however, believe that this reduction was sufficient to produce the observed temperature trends. Finally, many authors have attributed the rapid drop in tropospheric temperatures during the middle 1960s to the eruption of Agung in 1963.

These theoretical and empirical studies have only dealt with the most general aspects of the problem and many questions remain unanswered. How do the effects of eruptions occurring at different latitudes and at different times of the year differ? What are the spatial patterns of climatic change following individual eruptions and accompanying longer term fluctuations in the frequency of explosive volcanic activity? Finally, how can the effects of an individual eruption be distinguished in the

'noisy' climatic record? The year-to-year variability of climate is such that the effects of any one eruption, unless it is of a very great magnitude, are unlikely to be readily apparent.

It is therefore difficult to be precise about the climatological significance of the Mount St. Helens events. Reliable estimates of the volume of material ejected are not yet available. First estimates suggest that from 3 to 7 km³ of material was removed from the mountain in the eruptions that occurred in May 1980, but it is not clear how much of this material reached the stratosphere. It is believed, however, that a significant amount did penetrate the tropopause. On the basis of present evidence, it appears that of the major middle and high latitude eruptions this century probably only that of Katmai (Alaska, 1912) was much larger, although estimates of the volume of material ejected by that event vary widely. The first Mount St. Helens eruption of the 18th May appears to have been of the same order of

magnitude as that of Ksudatch (Kamchatka, 1907) and Bezymianny (Kamchatka, 1956). The eruption of Agung was of the same magnitude, but was probably more significant climatologically because of its equatorial position. Thus the eruption of Mount St. Helens could well prove to be among the most powerful of this century. After the explosive eruption of Mount St. Helens in 1842, three other eruptions occurred in the Cascade Mountains during the following thirteen years; Mount Rainier (1843), Mount Baker (1854) and Mount Hood (1854).

While we cannot predict the climatic effects of the recent eruptions with any certainty, it is likely that the dust veil will have noticeable optical effects over coming months. A brilliant red glow may be observed lingering near the horizon for some time after sunset, and a corona, or Bishop's ring, may be seen surrounding the sun. These effects were last widely seen after the eruption of Mount Fuego (Guatemala, 1974). In the past, extensive dust veils have given the sky a milky-white appearance for many months over large areas of the globe. It has even been suggested that similar optical effects

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influenced the painting of the English artist, Turner.

One of the major problems in determining the climatic impact of past eruptions has been a lack of firm data. There is insufficient information concerning the amount and composition of the material injected into the stratosphere by previous major eruptions. It is also only in recent years that reliable observations of the atmosphere, and particularly the upper layers, have become readily available. The catastrophic eruption of Mount St. Helens affords an important, first opportunity to study in detail the physics, chemistry, meteorology and climatology of the impact of a major middle latitude explosive event. □

The advantages of being evergreen

from Peter Moore

THE evergreen habit in plants has a number of obvious advantages, such as the conservation of energy and the capacity to indulge in photosynthesis over a considerable surface area whenever conditions are suitable. But there are some, rather contrasting, habitats in which the advantages may be more subtle. Take, for example, bogs and Mediterranean type heaths (chaparral or maquis). These two habitats are at opposite ends of the water supply spectrum, yet both are characterized by a flora in which evergreens are particularly prominent.

Since many evergreen plants are also xeromorphic, their association with wetlands is particularly intriguing. It is possible that the low temperatures and anaerobic conditions associated with bog peats results in a physiological drought even where water is apparently abundant, but an alternative and attractive explanation was put forward by Small (*Can. J. Bot.* **50**, 2227; 1972). He approached the problem from the point of view of the efficiency of nutrient utilization, for this is often a limitation to plant growth on bog peats. As a measure of efficiency in nutrient use, he took the length of time a unit of nitrogen remains in the plant before being lost by leaf fall. On this basis he found that deciduous bog plants performed about 60% more photosynthesis per unit nitrogen residence time than non-bog deciduous species, but that values for bog evergreens were 235% higher than for the bog deciduous species. Here, in the realm of nutrient conservation, could be the secret of the bog evergreen's success.

Small's Canadian study site had a very limited growing season, and this could have

affected the conclusions, but this does not apply to a study conducted in a swamp in Georgia by Schlesinger and Chabot (*Bot Gaz* **138**, 490; 1977). They compared the mineral nutrition and water relations of selected evergreen and deciduous swamp plant species and they found no evidence of water stresses developing, (the lowest leaf water potentials observed were -12.5 bars), thus eliminating the physiological drought hypothesis for this site. Leaf longevity on the part of the evergreen, however, could once again form the basis of a higher efficiency in the utilization of the limited nutrient resource. Although the nutrient and carbon costs in producing a leaf were greater for the evergreen, this was more than compensated for by its longer working life.

It is possible that evergreens also make better use of the available nutrients in chaparral habitats. Here the normal climate consists of cool, wet winters and hot, dry summers, and the bulk of plant growth occurs in spring. Litter falling in the dry season is thus subjected to leaching by the winter rains prior to the demand imposed by the spring flush of growth. Mooney and Rundel (*Bot Gaz* **140**, 109; 1979) have examined the pattern of nutrient uptake in the evergreen shrub, *Adenostoma fasciculatum* in the chaparral of California, and they find that there is an increase in the content of nitrogen and phosphorus as well as in the biomass of old

leaves during the winter period. This occurs prior to any new leaf production in the spring and it suggests that the old leaves are being used as a nutrient reservoir in which a nutrient capital can be accumulated during the winter dormancy. This is an efficient nutrient conservation technique since it avoids the winter leaching losses and it places the plant in a strong position for rapid growth when nutrient demand rises in the spring.

The long-lived, evergreen leaf may thus play an important role in the annual movement and storage of nutrients in the plant. Indeed, this value of the evergreen leaf may be prolonged beyond annual events. Rundel and Parsons (*Amer. J. Bot.* **67**, 1980) have conducted long term analyses of the foliage of *Adenostoma* and of *Ceanothus leucodermis* in California, and they find that the highest observed leaf concentrations of nitrogen, phosphorus and potassium in these species are in the years immediately after a fire, when there is a surplus of these elements in the soil. After about six years the foliar concentrations have fallen by up to 30% and they continue to decline very slowly over the next 60 years. The evergreen leaf, therefore, can provide a storage organ for excess nutrient elements which act as a reservoir for rapid further foliage growth over the four years following a fire. It seems that the longevity of the leaf is the essential feature which allows it to assume this function. □

Human babesiosis

from F.E.G. Cox

THE babesias are small intraerythrocytic protozoa, common in many mammals, some of which are important pathogens of cattle, horses, sheep and dogs. Several species occur in rodents and two, *Babesia microti* and *rodhaini*, are easily transmitted to mice and rats and have been extensively studied in the laboratory. From time to time, babesia-like parasites have been seen in smears of human blood but, because of their morphological similarity to malaria parasites, their significance has not been appreciated. Over recent years, however, human cases have become increasingly well documented and it is now possible to assess the importance of these parasites in man.

The first human cases were all in splenectomised individuals in Europe and, because cattle babesias are well known, it was assumed, and later shown to be correct, that these were the sources of the human infections. This assumption coloured attempts to identify the parasites involved in later cases and the rodent babesia, *B. microti*, was ignored. *Babesia microti* is practically universally found in rodents and insectivores throughout the northern hemisphere and easily infects laboratory rodents and splenectomized

monkeys (Shortt & Blackie *J. Trop. Med. Hyg.* **68**, 37; 1965) which the cattle species do not. *Babesia microti* was implicated as a cause of human babesiosis in the 1970s when a number of cases were recognized in the Nantucket Island area of the US and the parasite was isolated and transmitted to laboratory rodents.

The current status of human babesiosis has now been clarified and it seems that there are two quite distinct forms, the European, in splenectomised individuals and the American, in people with spleens. In the United States, there have been a number of serologically positive cases and 21 parasitological diagnoses, 19 of which definitely harboured *B. microti*. (Ruebush *Trans. R. Soc. Trop. Med. Hyg.* **74**, 149; 1980) and none of which had been splenectomised. This infection is mainly limited to the islands off the New England coast where the wild reservoirs of *B. microti* are the deer mouse, *Peromyscus leucopus*, and the meadow vole, *Microtus pennsylvanicus*. The vector is a deer tick, *Ixodes dammini* (Spielman *et al. J. Med. Ent.* **15**, 218; 1979) which feeds in its larval and nymphal stages on the deer mouse and meadow vole. No babesias have been

found in the deer on which the ticks also feed (Piersman *et al.* *J. Med. Ent.* **15**, 573; 1979) and the fact that isolates of *Babesia* from wild mice and voles resemble the human isolates using immunological criteria (Benach *et al.* *Amer. J. Trop. Med. Hyg.* **28**, 643; 1979) confirms that these rodents are the sources of the human infections. In Mexico, an unidentified babesia that infects hamsters has been isolated from three asymptomatic individuals (Osorno *et al.* *Vet. Parasit.* **2**, 111; 1976).

In Europe, the human cases now total 6; 2 from Yugoslavia, 2 from France and one from each of Ireland, Scotland and the USSR. In all cases the infected individuals had been splenectomised and all but the two from France died. The parasites implicated are *B. bovis* and *B. divergens* in Yugoslavia, *B. divergens* in Ireland and Scotland and one of the French cases, *B. bovis* in the USSR and the identity of the parasite in the remaining French case is unknown (Garnham *Trans. R. Soc. Trop. Med. Hyg.* **74**, 153; 1980). In the Scottish case the parasite has been returned to cattle and also passaged into gerbils, the first time

a rodent has been successfully infected with a cattle piroplasm. (Lewis & Williams *Nature* **278**, 170; 1979). The vector of human babesiosis in Europe has not yet been identified but *Ixodes ricinus* must be a possibility (Donnelly *Trans. R. Soc. Trop. Med. Hyg.* **74**, 158; 1980). It is unlikely that *B. microti* will infect man in Britain where the vector is *I. trianguliceps* which seldom, if ever, feeds on man.

Human babesiosis, then, occurs in two distinct forms. The American form, which is relatively avirulent, occurs in intact individuals and is caused by *B. microti* from rodents and the European form, which is usually fatal, occurs in splenectomised individuals and is caused by *B. divergens* or *B. bovis* from cattle. All these cases can be regarded as accidental infections of man acquired by farmers, campers and others likely to be bitten by ticks. The infection is characterised by fevers and anaemia with small rings resembling malaria parasites in the blood but the parasites are not killed by chloroquine.

This is a rare but interesting disease and is the latest addition to the growing list of zoonoses, those parasites transmissible from wild or domesticated animals to man. □

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Human interferon gene sequences

from Michael Houghton

THE excitement that has been generated recently by human interferon studies is continuing with the elucidation of the gene sequences (and hence polypeptide structures) of human fibroblast (F-IF) and Leukocyte (Le-IF) interferons. The two molecules are currently receiving much attention over their potential as *in vivo* anti-viral and antitumour agents (Stewart *The interferon system* Springer, Berlin, 1979).

Although both molecules seem to induce similar biological effects, the target cell specificities differ and antibodies raised against one type do not neutralise the other (Stewart *op cit.*). Also, there is some evidence that the two proteins are encoded by different mRNA molecules (Cavaliere *et al.* *Proc. natn. Acad. Sci. U.S.A.* **74**, 3287; 1977). The origins of these differences have become clearer now that sequences are available from both F-IF (Taniguchi *et al.* *Gene* **10**, 11; 1980; Houghton *et al.* *Nucl. Acids Res.* **8**, 1913; 1980; Derynck *et al.* *Nature* this issue, p542) and Le-IF (Mantei *et al.* *Gene* **10**, 1; 1980).

The F-IF mRNA contains 836 nucleotides (excluding the 3' poly A tail and the possible 5' cap structure) consisting of 72 and 203 nucleotides in the presumed 5' and 3' untranslated regions respectively, 63 nucleotides coding for a hydrophobic pre-peptide signal sequence that is responsible for protein secretion, and 498 nucleotides coding for a further 166 amino acids. If the latter are all present in the mature protein,

then a polypeptide molecular weight of 20,000 daltons can be predicted, a value which is significantly higher than the 16,500 dalton species that is thought to represent the cleaved, but unglycosylated protein observed in a reconstituted cell-free system (Derynck *et al.*). Also, the native glycosylated form of F-IF has itself been estimated to be 20,000 daltons (Havell *et al.* *J. Biol. Chem.* **252**, 4425; 1977) and so at this stage, one cannot completely rule out the possibility of additional processing of F-IF besides the cleavage of the N-terminal signal sequence.

Le-IF mRNA contains around 865 nucleotides (excluding the 3' poly A tail and probably a small number of nucleotides at the 5' terminus that have been removed during cDNA preparation) of which 242 are located in the presumed 3' untranslated region. As in the case of F-IF mRNA, there seems to be a region coding for a pre-peptide signal sequence of either 15 or, more probably, 23 amino acids again followed by a coding sequence of 498 nucleotides corresponding to 166 amino acids in the mature protein, assuming no further processing. Interestingly, in view of the fact that the biologically active product of Le-IF genes cloned in bacteria seems to be sequestered into the periplasmic space (Mantei *et al.*), it would seem there is

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at least some recognition of the eukaryote signal sequence by the appropriate bacterial membrane receptors and possibly, processing enzymes.

A comparison of the gene and amino acid sequences of F-IF and Le-IF (T. Taniguchi *et al.* *Nature*, this issue, p547) reveals a number of interesting features. First, although there is an average homology of about 45% between the nucleotide sequences coding for amino acids, the longest common stretch is only 13 nucleotides indicating that the interferons are the products of two closely related genes and not the result of a differential splicing process on the pre-mRNA product of one common gene. Second, three (or four) main domains of homology can be discerned between the coding nucleotide sequences of the two interferons, a feature which is even more pronounced upon comparing the amino acid structures, having lined up the presumed initiator methionine codons. Clearly, it is possible to speculate that these domains are responsible for important common functions of the two proteins such as the binding to common cellular receptors (Wiranowska-Stewart *et al.* *J. Gen. Virol.* **37**, 629; 1977) and the anti-viral and anti-tumour activities. Third, the overall data clearly suggest that there was a common ancestral gene for these two interferons.

It is also revealing to compare the amino-terminal sequence and amino acid composition of a human lymphoblastoid interferon (Zoon *et al.* *Science* **207**, 527; 1980) with the corresponding Le-IF data. While the former is probably of the leukocyte type (see Paucker *The Interferon System. Texas Reports on Biology and Medicine* (eds. Baron & Dianzani) **35**, 23; 1977), it seems as if it is the product of a distinct, non-allelic gene. Indeed, a second Le-IF cDNA clone has been observed by Mantei *et al.* which appears different from the first in terms of its restriction enzyme map. However, its degree of relatedness to the latter and the human lymphoblastoid interferon remains to be seen.

We can now look forward to further results from cloning experiments (both cDNA and genomic cloning) to provide more valuable information on the number and arrangement of genes coding for a particular type of interferon and their relatedness to each other and other types of interferon genes. It may now also be possible to modify and re-arrange these genes using standard genetic engineering techniques and other methods (e.g. Muller *et al.* *J. Mol. Biol.* **124**, 343; 1978) to produce new interferons with some particular advantages in specificity and activity. Such studies could also help in the detailed assignment of structure to function. Clearly, the work discussed here signifies the start of an era from which the molecular mechanism of interferon action will hopefully be elucidated □

ARTICLES

Late Quaternary water exchange between the eastern Mediterranean and the Black Sea

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Sea of Marmara sediments provide an almost complete record of major palaeo-oceanographic events affecting the Aegean and Black Seas between the late Pleistocene and the Recent. Fluctuating patterns of water mass exchange, resulting from regionally important climatic oscillations, produced the marked differences in lithofacies mapped between the Sea of Marmara and the eastern Mediterranean and Black Seas.

THE Sea of Marmara in northern Turkey is an elongate, east-west trending, almost totally enclosed depression between Thrace to the north and Anatolia to the south. It lies between the Black Sea to the north-east and the Aegean Sea to the south-west (Fig. 1). Connections with the Black and Aegean Seas are restricted, involving two narrow and shallow straits: the NNE-SSW trending Bosphorus, 30 km long and locally as shallow as 35 m, at the north-east Marmara sector, and the longer (60 km) and somewhat deeper (50–60 m), NE-SW trending Dardanelles to the west. The 210-km long Sea of Marmara contains three relatively deep basins, respectively 1,097, 1,389 and 1,238 m from west to east, and is bordered to the south by a broad shelf¹.

The lithofacies of radiocarbon-dated cores (latest Pleistocene to Holocene) collected in the easternmost basin, called the Eastern Marmara Basin², are described here for the first time, and are compared with recently detailed lithostratigraphic sections of comparable age from the Black Sea³ and the eastern Mediterranean⁴. Marked differences in the facies sequences and particularly in the age of the dark, organic-rich Holocene sapropel layers are recorded: those in the Aegean accumulated at ~9,000–7,000 yr BP (ref. 5); those in the Black Sea at ~7,000–3,000 yr BP (ref. 3). The origin of the Mediterranean sapropels remains controversial and has been attributed to changes of evaporation relative to precipitation^{6,7}, marked increase of freshwater outflow from the Black Sea^{8–10}, and an increase in discharge from rivers surrounding the eastern Mediterranean, particularly the Nile⁴. All hypotheses applicable to both Mediterranean and Black Sea sapropels imply broad-scale climatic changes that induced marked regional density stratification of water masses, progressive depletion of dissolved oxygen, and formation of hydrogen sulphide^{11,12}; development of euxinic conditions led to preclusion of higher forms of life on the sea floor. The most recent Holocene sapropel in the Mediterranean formed during what most workers believe to be a warming trend¹³; its appearance throughout the Levantine and Ionian basins has been directly related to the depth of the shallow Bosphorus sill which controlled the westward overflow of fresh water from the Black Sea^{8–10}. In contrast, the age and stratigraphic position of the youngest Black Sea sapropel are apparently not as closely related to sill depth and eustatism. The difference in sill depth between the Bosphorus and Dardanelles, only ~10–15 m, is too small to explain satisfactorily the much younger age of the Black Sea Holocene sapropel.

We interpret here the sequence of events that probably induced differences in the timing of sapropel accumulation in Aegean and Black Seas, and also evaluate differences between Late Quaternary lithofacies sequences deposited in the two regions. Four University of Miami gravity cores recovered in

1965 (RV Pillsbury cruise P6507) in the eastern Sea of Marmara (Table 1) were used in this study. The investigation of these cores included X-radiography, radiocarbon dating and petrological examination of 44 core sample splits: grain-size analyses, total organic matter and calcium carbonate content, scanning electron microscopy (SEM) of silt-size fraction, X-ray diffraction of clay and silt-size fractions, and petrographic identification of both terrigenous and biogenic components of the sand (>63 µm) fraction. All data are summarized in tables available from the National Physical and Solar-terrestrial Data Center, NOAA, Code 621, Boulder, Colorado (Accession no. MGG-21025001). Simplified, dated core logs of the Marmara units based on these results, and well established stratigraphic sections from the Aegean and Black Seas, are shown in Fig. 2.

Marmara sequences and palaeo-oceanographic implications

The base of one core, P6507-G6, on the southwestern slope of the Eastern Marmara Basin, is dated at ~14,000 yr BP. This core was supplemented by two others (G7, G8) from the basin plain in which recovered sections were dated at ~5,000 and 5,500 yr BP; the base of a fourth core, G9, from the eastern slope of this basin, is dated at about 6,800 yr BP (Table 1). Although this region lies along the presently active North Anatolian Fault^{14,15}, it has been shown that structural displacement has been relatively minor during the short time span considered¹⁶. We thus assume that the major late Pleistocene to Holocene eustatic rise depicted on the sea-level curves^{17,18}, rather than tectonics, is responsible for the sequence of palaeo-oceanographic events observed in the sedimentary record in this region. Five major late Quaternary to Recent events are identified (Fig. 3).

The basal section of the Sea of Marmara core G6, nearly 14,000 yr BP (Fig. 2), consists of olive, generally structureless, clayey silts with minor amounts (<5%) of sand. Substantial amounts of iron sulphide occur in the sand fraction, some filling a few, poorly preserved and probably reworked planktonic and benthonic foraminiferal tests. Moderate proportions of disseminated gypsum crystals of coarse silt and sand size also occur; although an authigenic origin is not precluded, the distribution pattern of gypsum as viewed in X-radiographs is comparable with that found in some natural saline lakes¹⁹, but may also have resulted from the effects of later groundwater brines (L. A. Hardie, personal communication). Total organic matter ranges to 15%, and calcium carbonate content to 6%. This facies suggests deposition in anaerobic conditions in a lake, possibly saline, that was bordered to the south by a broad, subaerially

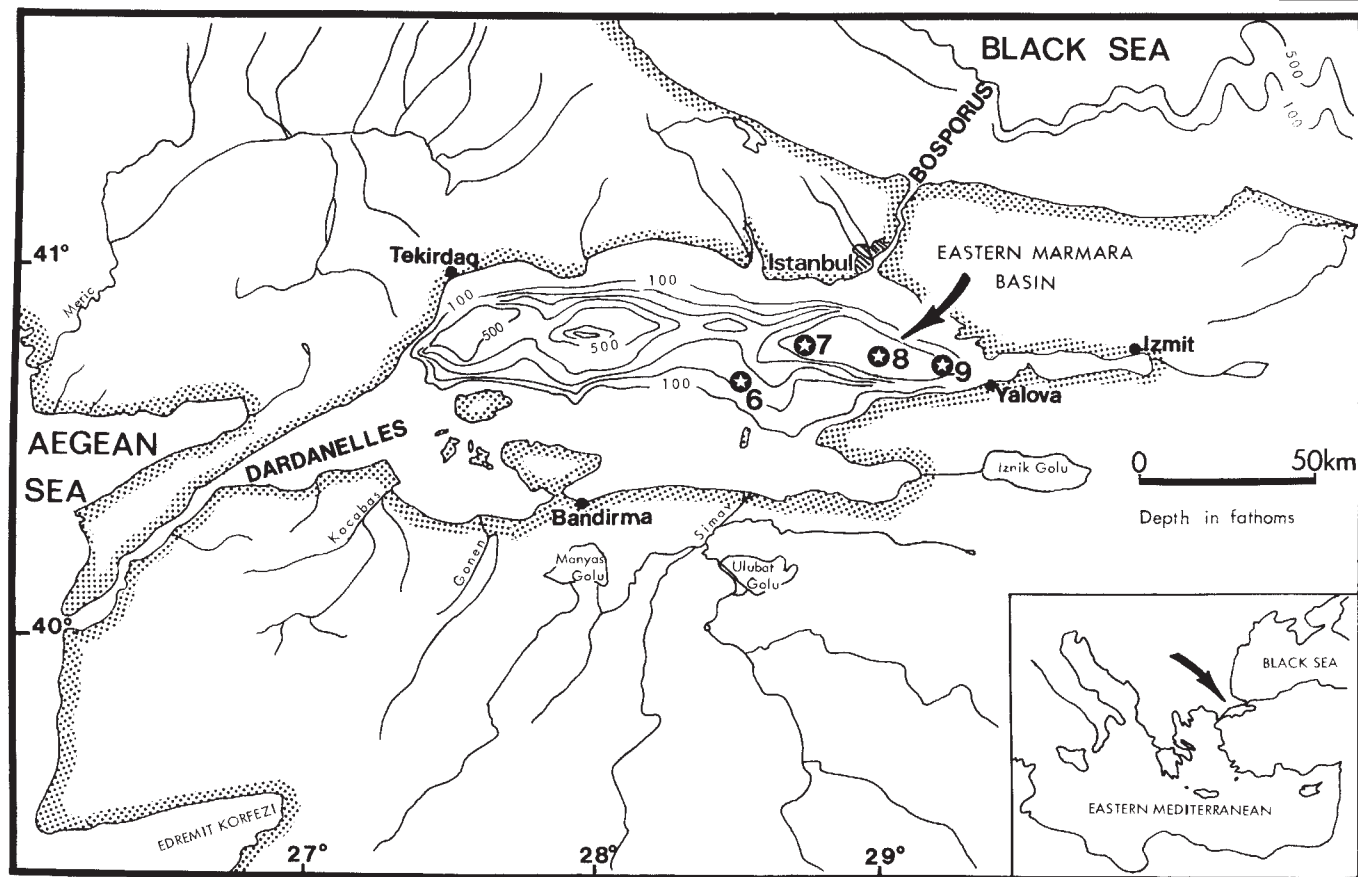


Fig. 1 Chart of the Sea of Marmara showing position of the cores discussed in the study.

exposed shelf. At this time of very rapid eustatic rise, Aegean sea level was at least 60m below the present stand according to widely used eustatic curves^{17,18}, and thus the Sea of Marmara was effectively blocked to the west by the Dardanelles. In contrast to the Marmara sequence, the typical Aegean Sea eastern Mediterranean facies during this period records deposition under open marine, vertically mixed and oxygenated conditions (Fig. 3 in ref. 5; Fig. 2 in ref. 4). At this time, the Bosphorus was probably a fluvial valley that separated the Marmara lake from the Black Sea¹⁶. The latter, in a lake phase, received meltwater from the receding Eurasian ice caps¹³, and thus displays freshwater characteristics; the Black Sea cores indicate that deposition occurred in conditions that intermittently involved sulphide formation and temporary anoxic settings³. Between the time of maximum eustatic low stand and ~12,000 yr BP, we postulate three totally separated seas, each with its distinct water mass configuration (Fig. 3a).

A marked change is noted in the core G6 section dated at ~12,000 yr BP (Fig. 2): the fine-grained sediment becomes dark olive-grey, contains an increased proportion of coarse silt, and displays poorly defined laminae. There is a sharp increase in large (1–2.5 mm), euhedral, well crystallized gypsum dominated by hemipyramidal faces (L. A. Hardie, personal communication); some crystals are coated with what is probably a bassanite-type ($\text{CaSO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$) dehydrate gypsum²⁰. Further change occurs in the overlying, somewhat younger sections (12,000–9,500 yr BP): gypsum crystals are translucent, and there is a sharp decrease in iron sulphide. A few planktonic tests include small forms of *Globigerina* cf. *G. atlantisae* Cifelli and Smith (R. Cifelli, personal communication), and rare coccoliths noted in SEM photographs. Total organic matter remains high (to 16%), but calcium carbonate content is reduced (to 3%). These characteristics suggest continuation of isolated lacustrine conditions with depleted dissolved oxygen and reduction

prevailing at the sampling site. Observed changes, however, signal an early, yet limited, introduction of water from basins adjacent to the Sea of Marmara. Aegean sea level at this time had risen to ~50 m below the present stand, sufficient to allow minor overflow of marine waters into the Sea of Marmara (Fig. 3b). Evidence is provided by the presence of low, but increased proportions of planktonic Foraminifera and nannofossils that could only have been transported from the west. The iron sulphide casts of some of these tests, associated with the dark colour of sediments, reflect continued anoxic bottom conditions resulting from maintenance of stratification at depth of Marmara water conditions. The Black Sea during the period from ~12,000 to 10,000 yr BP, still in its freshwater lake phase³, was receiving by way of major rivers (Danube, Dnepr, Dnestr, Dnepr and others) and also the Caspian Basin overflow¹³ a maximum quantity of meltwater from the receding European ice sheet. It has been suggested that this period was one of increased widespread wetness^{21,22}. These regionally important climatic influences resulted in a substantial rise of the Black Sea level and westerly-directed overflow of large volumes of water across both the Bosphorus and Dardanelles straits. Evidence of important Black Sea freshwater input to the eastern Mediterranean, coincident with possible changes in precipitation relative to evaporation, is recorded by the widespread presence of a grey, protosapropelic layer, usually 2–12 cm thick, that normally underlies the youngest (Holocene) sapropel in the eastern Mediterranean²³. Such protosapropels record the beginning of marked stratification and oxygen depletion in the Aegean and adjacent Mediterranean basins. The eustatic factor *per se* played only a minor role during this event of almost one-way flow from the Black Sea, across the Sea of Marmara, to the Aegean Sea (Fig. 3b).

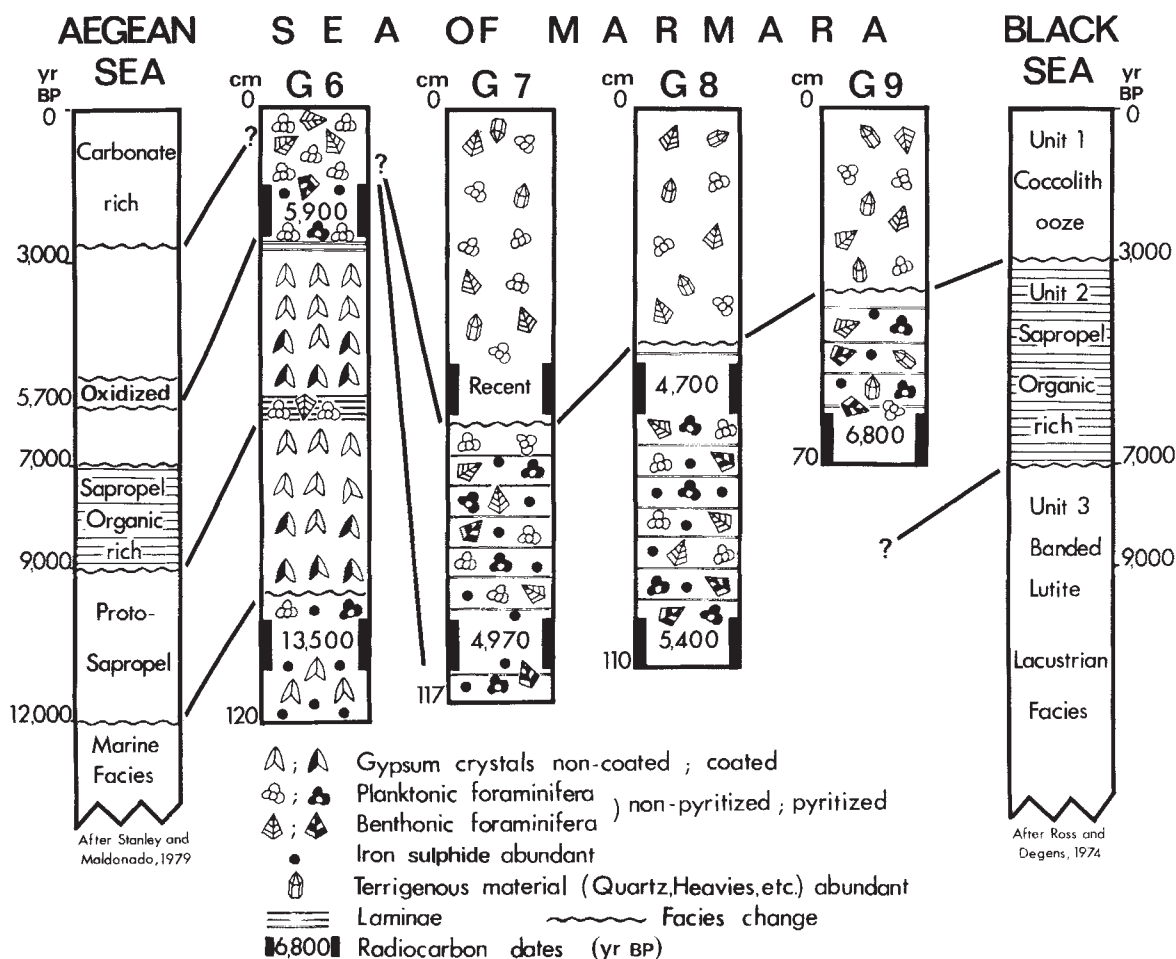
The G6 core section corresponding to ~10,000–9,000 yr BP (Fig. 2) is lighter in colour, displays fine laminae and contains

Table 1 Sea of Marmara RV Pillsbury cores examined in study (dates from Smithsonian Institution Radiation Biology Laboratory)

Core	Latitude	Longitude	Depth	Core length (cm)	Depth from core top (cm)	Age (yr BP)
P6507-G6	40°40.8'N	28°33'E	384 m (210 fm)	120	15-25 100-110	5,900 ± 130 13,530 ± 160
P6507-G7	40°45.5'N	28°46.5'E	1,193 m (650 fm)	117	50-60 100-110	Recent 4,970 ± 110
P6507-G8	40°44.5'N	29°01.8'E	1,209 m (660 fm)	110	50-60 100-110	4,755 ± 130 5,470 ± 125
P6507-G9	40°44'N	29°15.4'E	795 m (435 fm)	70	25-37 60-70	5,660 ± 130 6,800 ± 130

lower percentages of iron sulphide and total organic matter (to 15%) and slightly higher carbonate content (to about 4%). Although the proportion of microfauna (planktonic Foraminifera, nannofossils) remains low, a few open-marine benthonic Foraminifera (primarily *Bolivina* sp., *Fursenkoina* sp.) appear for the first time. The tests are well preserved without obvious dissolution features. Pyramidal gypsum crystals, larger than those in underlying sections, dominate the core section, and what is interpreted as a bassanite-type gypsum form reappears at ~9,000 yr BP, but is absent in younger overlying sections. Between 9,500 and 7,000 yr BP sediments are darker, richer in organic matter (to 18%), and show a decrease in iron sulphide, while benthonic

Foraminifera temporarily disappear. The continued eastward overflow of saline Mediterranean water across the Dardanelles spread along density interfaces in the eastern Marmara Basin, and water above the bottom remained partially, and at times completely, euxinic. The continued rapid eustatic sea-level rise during this period also enabled minor amounts of Mediterranean waters to cross the Bosphorus and overflow into the Black Sea³. The period between ~9,500 and 7,000 yr BP experienced a two-way flow regime above the Sea of Marmara, but the westward movement of fresh water into the Aegean and beyond clearly predominated (Fig. 3c). This resulted from continued, but reduced, influx of meltwater and fluvial discharge from the Danube and Russian rivers into the Black

**Fig. 2** Simplified logs of the Sea of Marmara (RV Pillsbury cruise P6507) core sequences, and correlation with stratigraphic sections in the Aegean⁴ and Black Seas³.

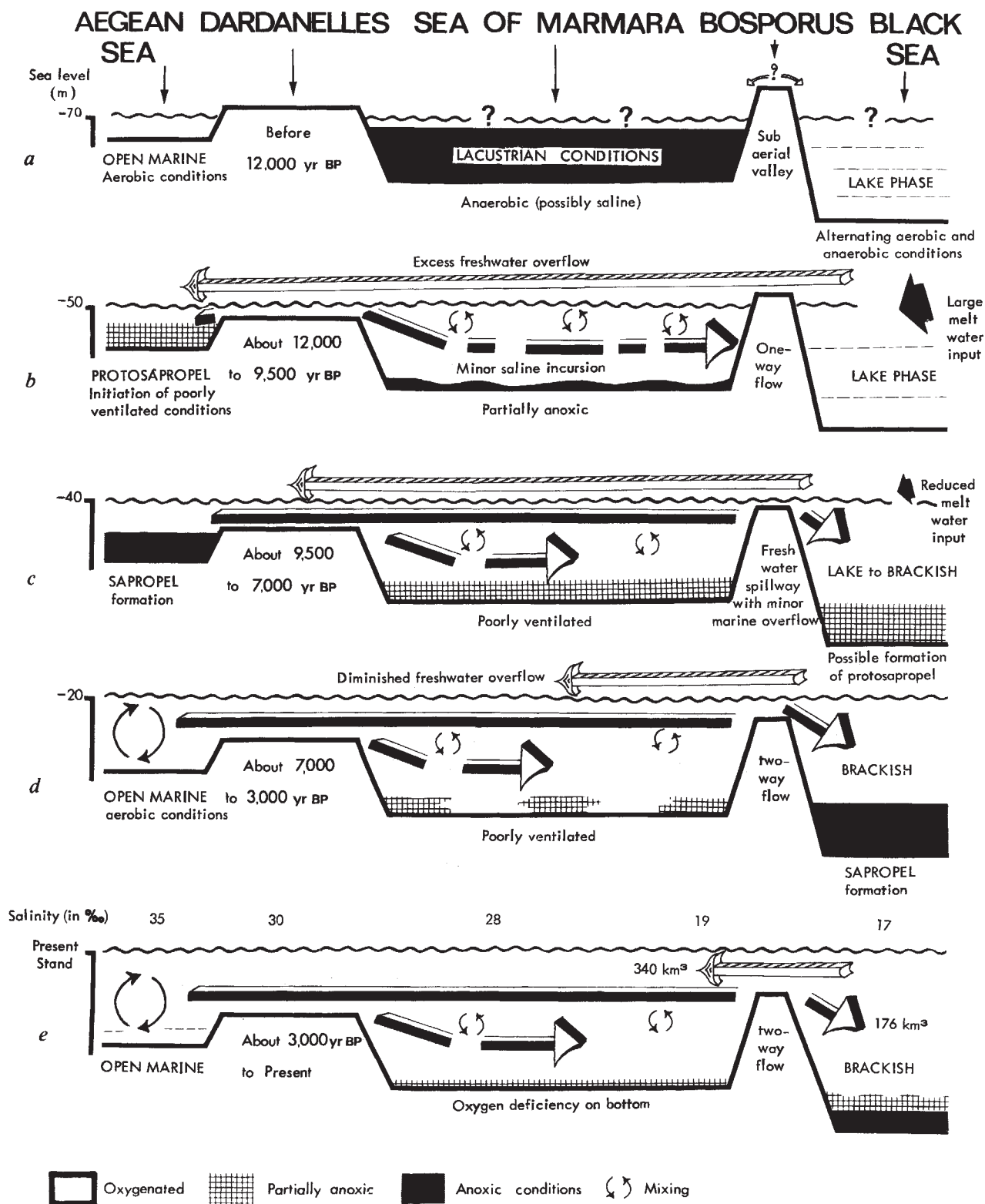


Fig. 3 Schematic depiction of oceanographic conditions in the Sea of Marmara region showing changes with time and the sequence of water exchange patterns between the Aegean and Black Seas. The shallower depth of the Bosphorus still is exaggerated, and the Marmara morphology much simplified, see text.

Sea, and apparently coincides with another short period of widespread wetness²¹. The powerful westward flow of Black Sea freshwater has been cited as a major factor in the formation of the most recent sapropel (9,500–7,000 yr BP) throughout most of the eastern Mediterranean Sea^{8–10}. Moreover, this discharge probably induced temporary current reversals and also the formation of grey reduced layers in the western Mediterranean^{9,24}.

A facies change occurs in core G6 at ~7,000–6,000 yr BP (Fig. 2). Sediments are lighter olive, coarser grained, include both well defined and vague laminae, and are characterized by the disappearance of gypsum crystals and slight diminution of total organic matter (to ~16%). Both planktonic and benthonic foraminiferal tests increase markedly (to ~30% of the sand fraction) and this is reflected by the higher percentage of carbonate content (to 6%). The planktonic foraminiferal population comprises only small tests of *Globigerina*, many of which are filled with iron sulphide. An essentially marine regime prevailed in the Sea of Marmara (Fig. 3d), and although not euxinic, the deeper parts of the basin remained oxygen-deficient. As a result of the continued but slower rise of sea level, increased amounts of Mediterranean water entered and influenced the formation of Black Sea sapropels during the period from ~7,000 to 3,000 yr BP (ref. 3) (Fig. 3). The reduced fluvial discharge into the Black Sea diminished the westwardly outflow of fresh water across the straits and the Sea of Marmara, allowing a return to vertical water mass circulation and oxygenated bottom water conditions as presently observed in the Aegean²⁵. A well defined oxidized layer, dated at ~5,700 yr BP and widely distributed throughout the eastern Mediterranean⁴ (Fig. 2), may be related to the proposed increased evaporation regime at 6,500–5,500 yr BP (ref. 21).

There is a good correlation among core G7, G8 and G9 lithofacies dated between ~6,000 yr BP and the present (Fig. 2). Sediments from ~5,000 to 3,000 yr BP are olive grey, laminated clayey silts with a high iron sulphide content. The sand fraction, ~5% of the total sediment, includes pelagic (dominant) and benthonic foraminiferal species similar to those of underlying sections. Small pelecypods and echinoderm fragments appear for the first time; these and sand-size terrigenous components, including light and heavy minerals, lithic fragments and plant matter, were probably displaced basinward from the Marmara shelf by turbidity currents. Some iron-stained quartz may also have a windblown origin (core G9). About 30% of the fauna displays an iron sulphide filling. Total organic matter ranges from 11 to 19%, and the calcium carbonate content does not exceed 5%. Radiocarbon dating of basin cores G7 and G8 reveals high sediment accumulation rates (to at least 70 cm per 1,000 yr) between 5,500 and 4,500 yr BP, indicating periodically intensified precipitation and increased discharge from rivers, primarily from the south, flowing into the Sea of Marmara.

At ~3,000 yr BP, Marmara sediments are darker olive and coarser grained (Fig. 2). Total organic matter remains high and calcium carbonate content low. Those layers in basin cores containing high proportions of terrigenous material of sand and silt-size were probably emplaced by turbidity currents. The marked increase in sediment accumulation rates at this time may be related to regionally important climatic fluctuations recognized in the eastern Mediterranean²⁶, or intensified tectonic activity¹⁵. This period coincides with the end of sapropel formation and the first major coccolithophore invasion in what had now become the brackish Black Sea³, and with carbonate-rich deposition that prevailed in the eastern Mediterranean⁴ (Fig. 2).

Conclusions

We postulate that the physical oceanographic conditions presently measured in the Sea of Marmara, including the two-way exchange of water masses through the Bosphorus and Dardanelles^{16,25,27}, were established at ~3,000 yr BP. The Sea of Marmara is presently characterized by salinity, temperature and chemical nutrient distribution patterns between those of the vertically circulated, well oxygenated marine Aegean (>35‰ salinity) and the brackish (<20‰ salinity) Black Sea (Fig. 3e). The low dissolved oxygen content in the Sea of Marmara (1.5 ml l⁻¹) and the high (to 14%) total organic content in surficial sediments result from continued stratification of water in this quasi-enclosed depression²⁷. The present environmental conditions are largely responsible for the restricted planktonic foraminiferal population (scarcity and small size of tests, few species).

We identify the Sea of Marmara as the easternmost branch of the Mediterranean, with the Bosphorus Strait serving as the major boundary with the Black Sea. Lithostratigraphic sequences deposited in the Sea of Marmara during the latest Pleistocene to the present include facies which typify deposition in almost continuously restricted circulation regimes. These sequences provide an almost complete record of palaeo-oceanographic events because of the quasi-enclosed physiographic configuration and intermediate position of this catchment basin between the larger seas. The dated Sea of Marmara facies offer a means to correlate more precisely sequences between the eastern Mediterranean and the Black Sea, and to explain lithologic differences in terms of fluctuating water mass exchange patterns resulting from regionally important climatic oscillations.

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Isolation and structure of a human fibroblast interferon gene

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Chimaeric plasmids containing double-stranded cDNA copies of mRNA induced in human fibroblasts by poly I·C were screened by an RNA selection method. A series of clones to which human fibroblast interferon mRNA selectively hybridized was identified. From the nucleotide sequence of the gene, the complete amino acid sequence of human fibroblast interferon was deduced. The protein is 166 amino acids long and is preceded by a 21-amino acid signal sequence.

At least three interferons with different antigenic specificity have so far been recognized. Leukocyte interferon, lymphoblastoid interferon and fibroblast interferon represent the type I interferons, whereas mitogen-induced immune interferon is a type II interferon (see ref. 1 for review). Although the structure of leukocyte and fibroblast interferons may be sufficiently similar for them to be recognized by the same receptor², there is evidence that they are encoded by different genes³. Biologically, they have a different host-cell range¹, different dose-response curves when assayed for antiviral activity on cell culture^{4,5} and a varying degree of cytostatic action in several types of cells^{6,7}. Both interferons have immunomodulating activities, both are able to activate 'natural killer' cells⁸⁻¹⁰ and both have promising clinical antiviral and antitumour activities¹¹⁻¹⁵.

The differences and similarities in activity of the two interferons will be better understood when their structures are known. All that is known so far of the structure of HF-IF (human fibroblast interferon) is the sequence of the 13 NH₂-terminal amino acids¹⁶ and the fact that it is a glycoprotein of about 20,000 molecular weight (MW)¹⁷⁻²⁰.

Two laboratories have recently used recombinant DNA technology to clone interferon cDNA^{21,22}. We have been working along similar lines, and here describe the construction and isolation of hybrid plasmids containing cDNA of HF-IF. The nucleotide sequence of the mature mRNA is derived from the analysis of the cloned DNA. On the basis of this information, the total amino acid sequence of HF-IF is deduced.

Isolation of RNA and construction of chimaera plasmids

Total RNA was isolated from VGS cells, a human diploid fibroblast cell line²³. The cells were primed with homologous human fibroblast interferon, induced for interferon production with poly I·C and superinduced with the antibiotic cycloheximide. Induction by poly I·C has been shown to induce only HF-IF in human fibroblasts, in contrast to viral induction, which results in a certain level of leukocyte interferon production²⁴. Confluent VGS fibroblasts passage 23-26 (usually 20 roller bottles, 670 cm² each) were primed with 100 units ml⁻¹ HF-IF for 16 h (ref. 23), and induced with 100 µg ml⁻¹ poly I·C in the presence of 50 µg ml⁻¹ cycloheximide for 4 h (ref. 25). The cells were scraped off, lysed with NP40, and the total cytoplasmic RNA was extracted with phenol. Usually 60-80 µg of total RNA was obtained from a confluent roller bottle. Purification over oligo(dT)-cellulose yielded polyadenylated mRNA representing 3-5% of the total RNA. This mRNA fraction was further enriched for interferon

mRNA by sedimentation in a 5-20% sucrose gradient in 50% formamide²⁶. Alternatively, the mRNA fraction was separated on a polyacrylamide gel and the fractionated RNA eluted from 2-mm gel slices²⁷ (the average decrease in mRNA length per successive gel slice was about 45 nucleotides). In all cases, the presence and amount of interferon mRNA were monitored by translation in *Xenopus laevis* oocytes²⁸, followed by assaying of interferon activity, based on reduction of the cytopathogenic effect caused by vesicular stomatitis virus (VSV). The use of human fibroblasts trisomic for chromosome 21 gave a 5-10-fold enhanced sensitivity to HF-IF in comparison with the diploid human fibroblasts²⁹. The peak fractions of these gradients were further used for the synthesis of double-stranded cDNA and cloning. The *in vitro* synthesis of cDNA was carried out by sequential treatment with avian myeloblastosis virus (AMV) reverse transcriptase, RNases T1 and A, *Escherichia coli* DNA polymerase I and S₁ nuclease, essentially as described elsewhere³⁰. This double-stranded cDNA was fractionated by polyacrylamide gel electrophoresis. The full-size interferon double-stranded cDNA was considered to be about 850 base pairs long on the basis of a prominent band on polyacrylamide gel with cDNA synthesized from the active mRNA, which was itself narrowly sized by gel fractionation (see above). This size estimate agrees with the sedimentation value of interferon mRNA in sucrose gradients³¹. The eluted double-stranded cDNA was elongated with homopolymeric (dT)-tails by terminal deoxynucleotidyl transferase. This tailed insert DNA was annealed to a double molar concentration of pBR322 plasmid DNA that had been cleaved in the β -lactamase gene by PstI restriction endonuclease and treated with terminal transferase to add homopolymeric (dA)-tails at the cleavage sites³⁰. The annealed hybrid plasmids were introduced into competent *E. coli* K12 HB101 cells³². About 97% of the tetracycline-resistant transformants were sensitive to carbenicillin, suggesting the presence of an insert cDNA in the PstI site of the β -lactamase gene. In this way, a library of about 17,000 transformants was constructed using 270 fmol (128 ng) of tailed insert DNA.

Identification of clones containing human interferon cDNA

An RNA selection method was used to identify clones with an HF-IF cDNA insert. Plasmid DNA from the clones was coupled to diazobenzoyloxymethyl (DBM)-cellulose powder³³. Total RNA from induced VGS cells was hybridized to the DNA and the hybridized mRNA was eluted in 99% formamide³⁴. The plasmid pSTNV-1 DNA, a pBR322

derivative containing the double-stranded cDNA from satellite tobacco necrosis virus (STNV) RNA³⁵, was added to the plasmid DNAs to be coupled; as the hybridization reaction was carried out in the presence of STNV RNA, we could evaluate the efficiency of hybridization, the extent of RNA degradation throughout the procedure and the possible presence of translational inhibitors. Half of the recovered RNA fractions were translated in the nuclease-treated rabbit reticulocyte lysate³⁶, followed by immunoprecipitation of the *in vitro* synthesized STNV coat protein and analysis on polyacrylamide gel (data not shown). The other half of the non-hybridized or hybridized RNA was assayed for the

presence of interferon mRNA by translation in *Xenopus* oocytes followed by the antiviral interferon assay.

Twelve groups of 46 clones from the library were screened for the presence of an interferon cDNA-containing plasmid (Table 1). One of the two which showed a positive response was subdivided into eight groups. One of these showed a clear positive interferon response after translation of the hybridized RNA. The individual clone containing the interferon cDNA was finally detected using DBM-cellulose paper disks, allowing elution with water at 80 °C and thus reducing the RNA degradation. The bacterial clone identified in this way is designated G-HB101-pHFIF-1, the plasmid being referred to as pHFIF-1.

Other clones containing double-stranded interferon cDNA were detected using the colony hybridization technique originally described by Grinstein and Hogness³⁷, as modified by Hanahan and Meselson (personal communication). Restriction enzyme analysis of pHFIF-1 revealed an internal *Hinf*I fragment of about 170 base pairs. This fragment was ³²P-labelled by nick translation³⁸ and the probe was used for screening part of the library for clones related to pHFIF-1. In this way, several additional clones were selected, their plasmids being designated pHFIF-2 to pHFIF-13. In our collection an average of 1% of the clones hybridized to the 170-base pair fragment of pHFIF-1. As the mRNA used for the cloning was 20–40-fold enriched by formamide-sucrose gradient centrifugation, one may estimate that approximately 1 out of 2,000–4,000 mRNAs is HF-IF mRNA under the induction regimen used for the VGS fibroblasts. That the secondary clones, detected by colony hybridization, actually contained interferon-specific sequences was further supported by the observation that plasmid DNA of one of these clones, pHFIF-2, was equally effective in selecting interferon mRNA (data not shown). Furthermore, the mRNA hybridized to either pHFIF-1 or pHFIF-2 gave rise to the same polypeptide of about 18,500 MW after translation in the rabbit reticulocyte lysate and immunoprecipitation with antiserum against partially purified HF-IF (Fig. 1). Addition of a microsomal fraction of dog pancreas to the reticulocyte lysate³⁹ produced two modified forms of this polypeptide, one with an apparent MW of ~20,500 and the other ~16,500, the former corresponding in size to HF-IF secreted from induced culture cells^{17–19}. The protein of lower MW suggests maturation by cleavage of a signal peptide, as will be discussed below.

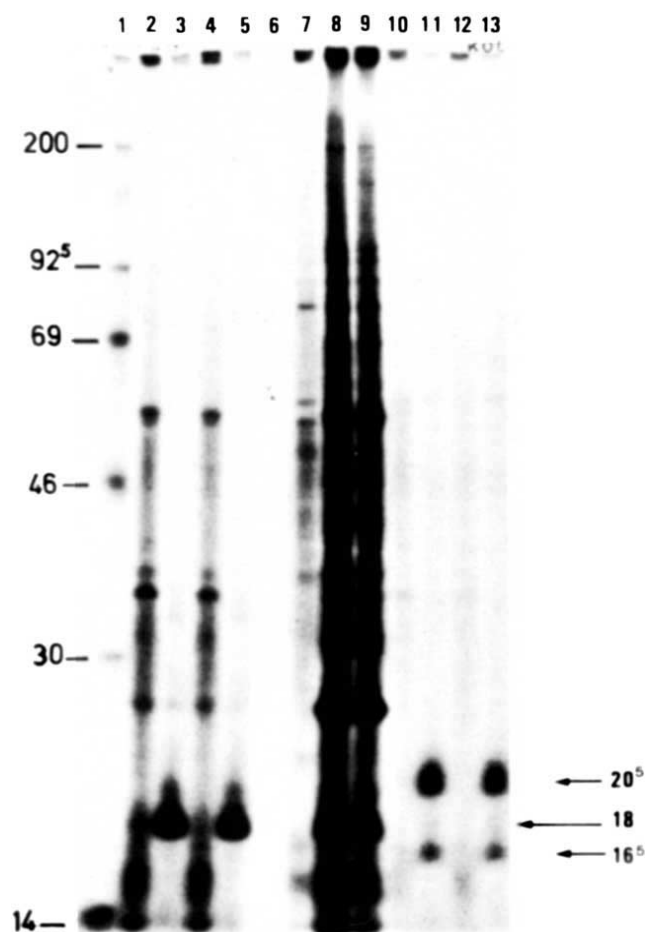


Fig. 1 Polyacrylamide gel electrophoresis of cell-free translation products from HF-IF mRNA after hybridization on different plasmid DNAs. Samples (30 µg) of total induced VGS-RNA were hybridized³⁴ to DBM-cellulose filters containing 3 µg DNA from pHFIF-1 or pHFIF-2. All RNA samples were divided into two equal parts and translated for 45 min at 31 °C in 25-µl reaction mixtures containing 1.7×10^7 c.p.m. ³⁵S-methionine (~1,000 Ci mmol⁻¹) and 150 µg ml⁻¹ calf liver tRNA in a micrococcal nuclease-treated rabbit reticulocyte lysate³⁶ in the absence (lanes 2–9) or presence (lanes 10–13) of 6.4_{280} ml⁻¹ dog pancreas microsomes³⁹. The reaction products were precipitated by addition of 2 µl of goat anti-HF-IF (200,000 HF-IF neutralizing units ml⁻¹) in the presence of 1% sodium deoxycholate and 1% NP40 for 1 h at 37 °C and 30 µl of a 10% suspension of *Staphylococcus aureus* Cowan I for 30 min at 20 °C. After extensive washing, the bacterial pellet was resuspended and boiled for 2 min in electrophoresis sample buffer, clarified for 2 min at 9,000g and applied to a 13% polyacrylamide gel. After electrophoresis, the gel was treated with Enhance (NEN) and fluorographed for 5 days at -70 °C. Lane 1, ¹⁴C-protein markers (Radiochemical Centre); the MW of the proteins are indicated at the left; lanes 2 and 10, pHFIF-1, non-hybridized RNA; lanes 3 and 11, pHFIF-1, hybridized RNA; lanes 4 and 12, pHFIF-2, non-hybridized RNA; lanes 5 and 13, pHFIF-2, hybridized RNA; lane 6, endogenous activity of the reticulocyte lysate; lane 7, total RNA from non-induced VGS cells (2 µg); lanes 8 and 9, total RNA from induced VGS cells (2 µg). The estimated MWs of the HF-IF precursor (lanes 3, 5) and of its putative processed, non-glycosylated and glycosylated derivatives (lanes 11, 13) are indicated at the right.

Physical characterization and sequence analysis of the cDNA insert

The plasmid DNAs of a series of clones containing an interferon cDNA insert as detected by hybridization were further characterized using restriction enzymes. Most attention was given to pHFIF-1, 2, 3, 6 and 7. Using the detailed physical map of pBR322 (ref. 40), the insert length could be estimated, either by using enzymes which do not cleave the insert, such as *Msp*I (= *Hpa*II) and *Hha*I, or by summation of the length of different restriction fragments. The insert cDNA of pHFIF-1 was, on the basis of its length, considered to be incomplete. Three other plasmids, pHFIF-3, 6 and 7, all had an insert of more than 850 base pairs, approximately the expected size of a full-length cDNA. Although most restriction enzymes tested had the same number of cleavage sites in these DNAs, clear differences could be observed in the patterns of restriction fragments obtained. Detailed analysis revealed that some awkward inversions had occurred. The arrangement of these inversions all had a similar appearance (Fig. 2). In all three cases, only a single segment was transposed, for example, in pHFIF-3, the *Pst*I-*Hind*II segment is inverted. The arrangement was confirmed and shown by nucleotide sequence analysis always to involve a cross-over point in the 5'-untranslated leader sequence. A second cross-over could conceivably have occurred in the tail region but it is also

Table 1 Outline of the screening strategy used for detecting plasmids containing interferon-specific DNA

Library (17,000 clones)	→	Group (46 clones)	→	Subgroup (7 or 8 clones)	→	Individual clone
Plasmid DNA source		Plasmid DNA bound on DBM-cellulose		Interferon activity ($\log_{10}$ units ml^{-1})*		
				Non-hybridized RNA		Hybridized RNA
Group 0 (46 clones)		1 μg DNA per clone		0.2		0.5
Subgroup 0 ₁ (8 clones)		3 μg DNA per clone		0		1.2
				0		1.5
				0		0.5
Individual clone 0 _{1/8}		3 μg DNA per clone		0		1.0
(subsequently referred to as pHFIF-1)				0		1.7
				0		1.2

Two groups of 46 clones were chosen arbitrarily from a total library of 17,000 clones (see scheme at top of table). As an example, the results of a positive group (group 0), subgroup (three experiments) and individual clone (also three experiments) are given. Total cytoplasmic RNA (15 μg) from induced VGS cells was used for hybridization. The non-hybridized and hybridized mRNA fractions³⁴ were precipitated twice with ethanol, dissolved in 2 μl water and assayed for the presence of interferon mRNA as follows. Five *X. laevis* oocytes were injected with 50 nl per oocyte²⁸. After incubation, a homogenate was obtained by crushing the oocytes in 40 μl incubation medium and cleared by centrifugation at 9,000g for 2 min. Human fibroblast interferon was assayed by a CPE (cytopathic effect)-inhibition technique in human fibroblasts trisomic for chromosome 21 in microtitre trays. The cells were challenged with VSV (Indiana strain) and the CPE was recorded at 24 h. All assays included an internal standard of HF-IF which was itself calibrated against the NIH human fibroblast reference G023-902-527.

* The limit of detection is 0.1 $\log_{10}$ units ml^{-1} .

possible that only a single cross-over was necessary to generate the inversion. The actual reason for this phenomenon is unclear; presumably it is a cloning artefact, although we have never seen this in our other work.

Restriction fragments labelled at the 5' end were prepared and sequenced, essentially according to Maxam and Gilbert⁴¹. The procedure used for *in vitro* DNA synthesis and cloning enabled us to determine the orientation of the coding strand. Indeed, the cDNA synthesis on the mRNA was initiated by a p(dT)₁₀ primer hybridized to the poly(A)-tail at the 3' end of the mRNA. This results in a (dA)₁₀ stretch at the 3' end of the (+)strand (the second DNA strand), followed by a dT-tract due to the elongation with terminal deoxynucleotidyl transferase. This feature allowed us to identify the 3' end of the coding strand in several plasmids examined.

Figure 3 shows the strategy for the sequencing analysis. All sequences were read several times, either on the same strand but from different restriction sites, or on the opposite strand. These results obtained on the series of clones allowed us to deduce the total nucleotide sequence of HF-IF cDNA.

The deduced primary structure of human fibroblast interferon mRNA and protein

The primary structure of the mRNA was determined on the basis of the DNA sequence and is shown in Fig. 4. The sequence we have deduced is 828 nucleotides long, excluding the poly(A) tail at the 3' end. One continuous reading frame was established, starting from the AUG codon at position 65 (immediately preceded by the *Hind*II site) and ending with the stop codon UGA at position 626, followed almost immediately by the single *Bgl*II restriction site. This region is 561 nucleotides long and thus is coding for 187 amino acids. The untranslated 5' end is 64 nucleotides long, but is presumably incomplete considering the method of *in vitro* synthesis of double-stranded cDNA. Although the total mRNA has an A + U content of 58% and the coding region an A + U content of 55% (normal values for a eukaryotic mRNA), the 5'-untranslated sequence is rather low in these bases—48% A + U. The 3'-untranslated region, excluding the poly(A) tail, is 203 nucleotides long and can be divided into two segments.

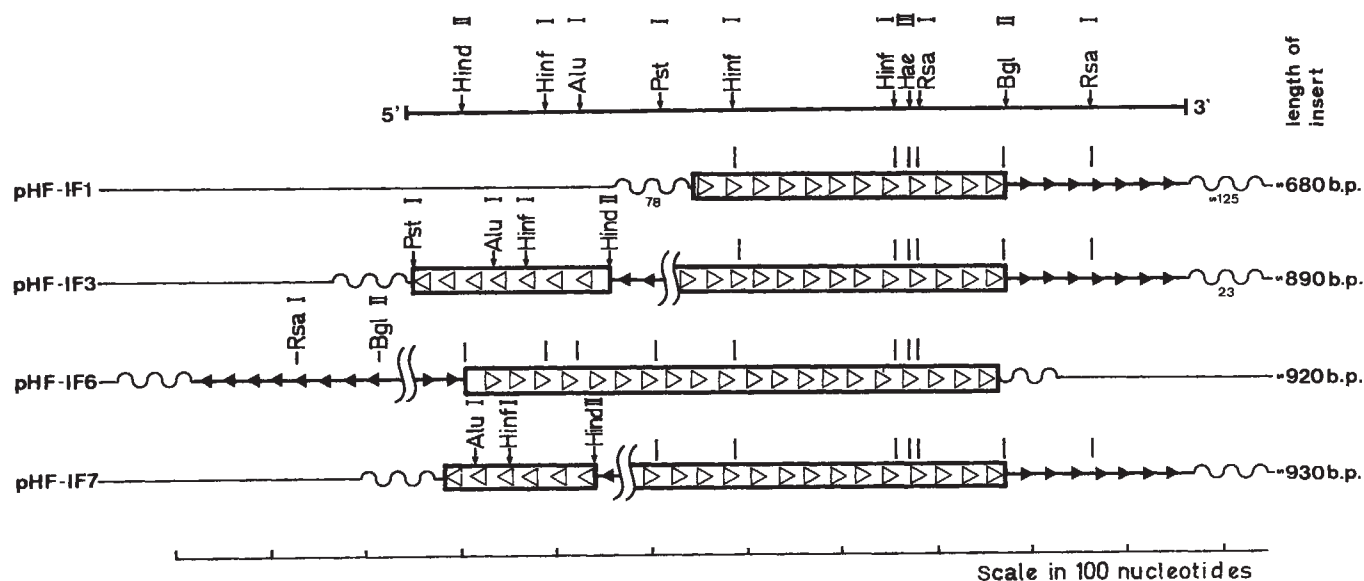
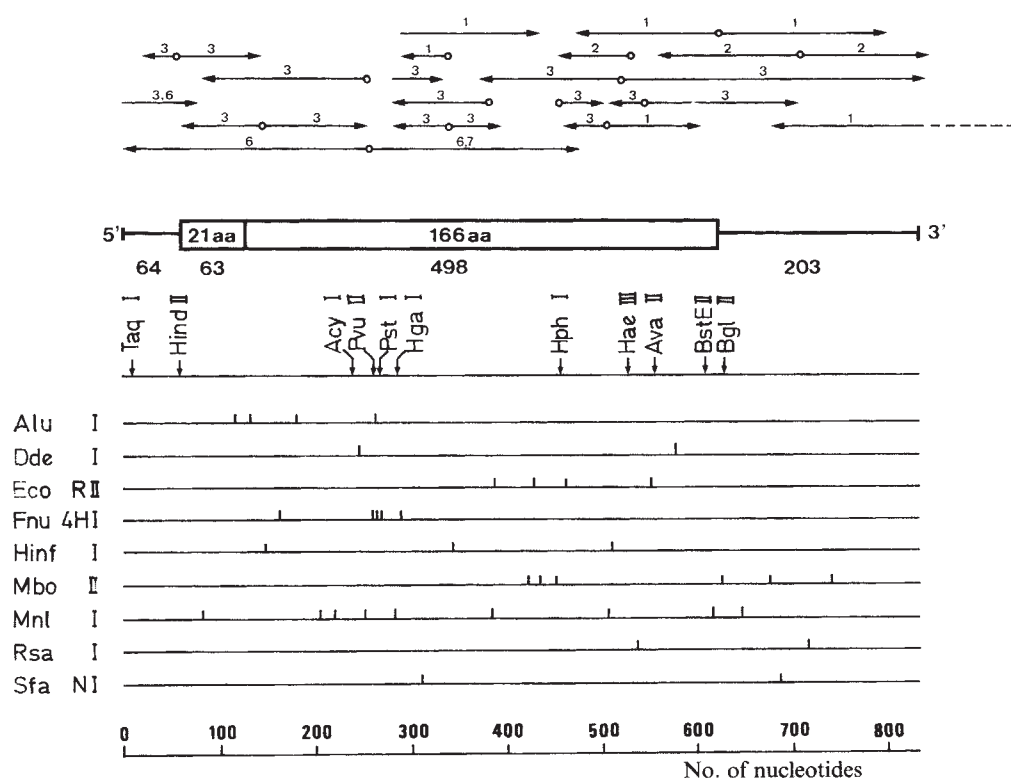


Fig. 2 Schematic representation of the organization of the cDNA inserts in pHFIF-1, 3, 6 and 7. The upper line represents a complete cDNA copy of interferon mRNA, with some reference restriction sites, and can be aligned with the more detailed map in Fig. 3. The dA-dT tails are indicated by a wavy line (the length, when known, is given underneath). The boxed segments indicate the translated sequence of the mRNA and the triangles indicate the 5'→3' direction of the coding strand of the insert DNA, in the translated region as well as in the 5' and 3' untranslated regions. An interruption in the base line of the maps indicates the cross-over regions of the inversion. b.p., Base pairs.

Fig. 3 Restriction map and sequencing strategy for the HF-IF cDNA gene. The central diagram shows the organization of the mRNA for human fibroblast interferon, with the 5'-untranslated region, the boxed translated sequence with the presumed signal peptide and the 3'-untranslated segment, the number of amino acids (aa) and nucleotides being indicated for each region. At the top, the regions covered by sequencing are indicated; the open dots correspond to the ^{32}P -labelled 5' ends. The numbers above the arrows refer to the different clones analysed. Using 0.5-mm gels, it was occasionally possible to read over 300 nucleotides from the labelled site. Below is a restriction endonuclease map of the double-stranded cDNA, constructed by computer search on the basis of the complete nucleotide sequence, many of these sites being experimentally verified. Enzymes cutting the double-stranded cDNA only once are indicated on the top line and enzymes cleaving at multiple sites are indicated individually below. Recognition sequences of restriction enzymes are listed by Roberts⁵⁶.



GCAA CCUUCGAAG CCUUGCUCU GGCACAACAG GUAGUAGGCG ACACUGUUCG UGUUGUCAAC **AUG** ACC, AAC, AAG, UGU, CUC, CUC, CAA, AUU, GCU, CUC, CUG, 100
 LEU-CYS-PHE-SER-THR-THR-ALA-LEU-SER **MET-SER-TYR-ASN-LEU-LEU-GLY-PHE-LEU-GLN-ARG-SER-SER** ASN-PHE-GLN-CYS-GLN-LYS-LEU-LEU-
 UUG, UGC, UUC, UCC, ACU, ACA, GCU, CUU, UCC, AUG, AGC, UAC, AAC, UUG, CUU, GGA, UUC, CUA, CAA, AGA, AGC, AGC, AAU, UUU, CAG, UGU, CAG, AAG, CUC, CUG, 190
 TRP-GLN-LEU-ASN-GLY-ARG-LEU-GLU-TYR-CYS-LEU-LYS-ASP-ARG-MET-ASN-PHE-ASP-ILE-PRO-GLU-GLU-ILE-LYS-GLN-LEU-GLN-GLN-GLN-
 UGG, CAA, UUG, AAU, GGG, AGG, CUU, GAA, UGC, CUC, AAG, GAC, AGG, AUG, AAC, UUU, GAC, AUC, CCU, GAG, GAG, AUU, AAG, CAG, CUG, CAG, CAG, UUC, CAG, 280
 LYS-GLU-ASP-ALA-ALA-LEU-THR-ILE-TYR-GLU-MET-LEU-GLN-ASN-ILE-PHE-ALA-ILE-PHE-ARG-GLN-ASP-SER-SER-SER-THR-GLY-TRP-ASN-GLU-
 AAG, GAG, GAC, GCC, GCA, UUG, ACC, AUC, UAU, GAG, AUG, CUC, CAG, AAC, AUC, UUU, GCU, AUU, UUC, AGA, CAA, GAU, UCA, UCU, AGC, ACU, GGC, UGG, AAU, GAG, 370
 THR-ILE-VAL-GLU-ASN-LEU-LEU-ALA-ASN-VAL-TYR-HIS-GLN-ILE-ASN-HIS-LEU-LYS-THR-VAL-LEU-GLU-GLU-LYS-LEU-GLU-LYS-GLU-ASP-PHE-
 ACU, AUU, GUU, GAG, AAC, CUC, CUG, GCU, AAU, GUC, UAU, CAU, CAG, AUA, AAC, CAU, CUG, AAG, ACA, GUC, CUG, GAA, GAA, AAA, CUG, GAG, AAA, GAA, GAU, UUC, 460
 THR-ARG-GLY-LYS-LEU-MET-SER-SER-LEU-HIS-LEU-LYS-ARG-TYR-TYR-GLY-ARG-ILE-LEU-HIS-TYR-LEU-LYS-ALA-LYS-GLU-TYR-SER-HIS-CYS-
 ACC, AGG, GGA, AAA, CUC, AUG, AGC, AGU, CUG, CAC, CUG, AAA, AGA, UAU, UAU, GGG, AGG, AUU, CUG, CAU, UAC, CUG, AAG, GCC, AAG, GAG, UAC, AGU, CAC, UGU, 550
 ALA-TRP-THR-ILE-VAL-ARG-VAL-GLU-ILE-LEU-ARG-ASN-PHE-TYR-PHE-ILE-ASN-ARG-LEU-THR-GLY-TYR-LEU-ARG-ASN
 GCC, UGG, ACC, AUA, GUC, AGA, GUG, GAA, AUC, CUA, AGG, AAC, UUU, UAC, UUC, AUU, AAC, AGA, CUU, ACA, GGU, UAC, CUC, CGA, AAC **UGA** AGAUCUCCUA GCCUG₆₄₃
 UGCCU CUGGGACUGG ACAAUUGCUU CAAGCAUUCU UCAACCAGCA GAUGCUGUUU AAGUGACUGA UGGCUAAUGU ACUGCAUUG AAAGGACACU AGAAGAUUUU GAAAU₇₅₃
 UUUUA UAAAAUUAUG AGUUUUUUU AUUUUUUUA AUUUUUUUU GGAA^{AAUAAA} UUAUUUUUGG UGCAAAAGUC AAAAAAAA_n ...

Fig. 4 Nucleotide sequence of the human fibroblast interferon mRNA and corresponding amino acid sequence. The nucleotide sequence is presented as the mRNA sequence, derived from analysis of the double-stranded cDNA in several plasmids; the actual mRNA may be longer at the 5' end. The initiation and termination codons are shown in heavily outlined boxes. The AAUAAA sequence, presumably present near the 3' end in all polyadenylated eukaryotic cellular mRNAs, is shown in a dashed box. The nucleotide numbering is given at the right. The nucleotide sequence is translated into an amino acid sequence consisting of a 21-amino acid signal peptide, from the initiation codon up to the vertical arrow, and the mature polypeptide. The boxed amino acid sequence, starting from the vertical arrow, indicates the NH_2 -terminal sequence derived from direct analysis of the protein¹⁶.

	U	C	A	G	
U	Phe { 4 5 Leu { 3	Ser { 1 1	Tyr { 4 6 Ochre Amber	Cys { 2 1 Opal Trp 3	U C A G
C	Leu { 3 6 2 10	Pro { 1	His { 3 2 Gln { 3 8	Arg { 1	U C A G
A	Ile { 5 4 2 Met 4	Thr { 2 3 2	Asn { 4 8 Lys { 4 7	Ser { 2 5 Arg { 5 5	U C A G
G	Val { 1 3 1	Ala { 2 3 1	Asp { 2 3 Glu { 5 8	Gly { 1 1 2 2	U C A G

Fig. 5 Codons used in human fibroblast interferon mRNA. The numbers refer to the frequency with which each triplet is used.

The region proximal to the UGA termination codon has a normal A+U content, whereas the distal portion is extremely A+U rich, a feature also observed, although not to this extent, in the mRNA of rat growth hormone⁴². Indeed, starting from position 744, only two C and eight G residues are present in a total of 84 nucleotides. A striking feature of this same region is the presence of many A(U)₂₋₅ stretches. The AAUAAA sequence presumably present in all polyadenylated eukaryotic cellular mRNAs and thought to be involved in processing or polyadenylation of the mRNA⁴³, is found some 20 nucleotides before the often observed C residue immediately preceding the poly(A) tail.

As in other vertebrate DNA and RNA sequences characterized so far, the dinucleotide CG is very rare in the mRNA for HF-IF. Only one of the six occurring CG dinucleotides is used within a codon (Fig. 5). This deficiency is reflected in the strong preference for AGG and AGA as codons for arginine, a preference which has also been observed in the genome of SV40 (ref. 44) and polyoma⁴⁵ and the haemagglutinin gene of a human influenza strain⁴⁶, but not, however, in the mRNA for rat growth hormone⁴² or human chorionic somatomammotropin⁴⁷. The apparently strong preference for CUG as codon for leucine, which seems to be a general phenomenon in eukaryotic cellular mRNAs but not in the mRNAs of SV40 (ref. 44) or polyoma⁴⁵ virus, is also found in HF-IF mRNA. There is also preference for AGU and AGC as codons for serine.

The amino acid sequence of HF-IF, deduced from the nucleotide sequence of the coding region, is also presented in Fig. 4. The 13 NH₂-terminal amino acids of mature HF-IF were recently directly determined by protein microsequencing techniques¹⁶. A corresponding nucleotide sequence begins at nucleotide 128; therefore, we conclude that this represents the NH₂-terminus of the mature HF-IF. It is preceded by a segment of 21 amino acids starting with an AUG at position 65. As interferons are secretory proteins, we postulate that this region is a signal peptide, cleaved off during or after transport of the nascent protein across the membrane. Indeed, it has been well established that the majority of secretory proteins start with a hydrophobic signal peptide which is subsequently removed⁴⁸. The hydrophobicity of this segment, mainly of the central part, and its length of 21 amino acids are consistent with the known properties of a signal peptide^{48,49}. Also, as generally observed^{48,49}, this signal peptide ends with an amino acid (serine in this case) of lower MW than the first NH₂-terminal amino acid of the cleaved protein. Direct evidence for this signal peptide has been obtained experimentally: addition of the microsomal fraction of dog pancreas to the *in vitro* translation mix, followed by immunoprecipitation with anti-interferon antiserum, results in a protein with an apparently lower MW than the unprocessed protein, indicating that a segment has been cleaved off (Fig. 1).

The mature HF-IF contains 166 amino acids on the basis of the deduced protein sequence. The amino acid composition is

in reasonable agreement with the composition as determined by direct analysis^{16,19}. It has a remarkably low content of proline and is very rich in the hydrophobic amino acids leucine and isoleucine, and also in tyrosine. The intrinsic hydrophobicity of HF-IF is well known and is also revealed by its interaction with several ligands⁵⁰. Three cysteines are present in HF-IF, which means that there must be at least one free thiol group.

The mature fibroblast interferon has been shown to be a glycoprotein^{17,19}. However, direct localization and characterization of the carbohydrate moieties on the polypeptide have not been described. Attachment of carbohydrate through N-glycosidic linkage is known to occur on the asparagine in the triplets Asp-X-Ser or -Thr, the presence of this sequence being a necessary but not a sufficient condition for glycosylation⁵¹. Only one asparagine allowing this type of glycosylation is found in fibroblast interferon, at position 80 of the amino acid sequence. Alternatively, or additionally, O-glycosidically linked oligosaccharides could be attached to serine and/or threonine residues, a good example of this being the human erythrocyte glycophorin⁵².

We are now in a position to reconstruct a plasmid with the total HF-IF genetic information under a prokaryotic transcription signal and to test for its expression in a bacterial system.

After submission of this manuscript, we identified a clone, pHFIF-21, having a full-length insert without rearrangements. Also we learned that Taniguchi *et al.*⁵³ have also determined the nucleotide sequence of a cloned HF-IF gene. The deduced amino acid sequence is identical to that reported here and shows the homology (see accompanying paper⁵⁴) with the sequence of human leukocyte interferon⁵⁵.

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Human leukocyte and fibroblast interferons are structurally related

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The coding sequences of the cDNAs of cloned human leukocyte interferon I and human fibroblast interferon show homologies of 45% at the nucleotide and 29% at the amino acid level. We conclude that the two genes were derived from a common ancestor.

THE acid-stable human interferons are subdivided into two major groups, fibroblast interferons (F-IF) and leukocyte interferons (Le-IF); these are the major components of the interferons produced by induced fibroblasts and leukocytes, respectively. Some cells, such as the lymphoblastoid Namalva cell line, produce a mixture of 90% Le-IF and 10% F-IF^{1,2}. The two interferon types have several features in common: both are glycoproteins with molecular weights ranging from 16,000 to 26,000 (refs 3-9), the induction and shut-off of their synthesis seem to be under similar control⁶, and at least some of the responses elicited in target cells are similar, such as induction of an antiviral state, which is accompanied by increased synthesis of several proteins¹⁰⁻¹³. Nonetheless, the two kinds of interferon differ in many respects. Antibodies directed against Le-IF do not neutralize F-IF and vice versa¹⁴, the target cell specificities of the two interferons differ¹⁵, and the sequences of the 13 amino-terminal amino acids of F-IF and Le-IF (from lymphoblastoid cells) show no homology^{16,17}. Although Le-IF and F-IF are encoded by different mRNA species¹⁸, it is not known whether these mRNAs are transcribed from distinct genes or whether they arise from the same gene through a common precursor which is processed or spliced in different modes.

We have recently cloned and sequenced one species each of Le-IF (Le-IF I)^{19,20} and F-IF cDNA^{21,22}. A second species of Le-IF (Le-IF II) cDNA has recently been identified (M. Streuli, S.N. and C.W., unpublished results).

Comparison of the amino acid sequences of Le-IF and F-IF

In Fig. 1 the nucleotide sequences of Le-IF I and F-IF cDNA were aligned so that the AUGs closest to their 5' termini coincided. From the amino-terminal sequence published for F-IF¹⁶ and lymphoblastoid Le-IF¹⁷, one can determine that for F-IF and Le-IF, respectively, the 21st and 23rd codons following the initiation triplet represent the first amino acid of the interferon polypeptide. Presumably, the stretch in between encodes a signal peptide. As the respective putative signal peptides of Le-IF and F-IF comprise 23 and 21 amino acids, the IF polypeptides, as aligned in Fig. 1, are shifted by two residues relative to their termini. In this alignment, 48 of 166 positions (29%) of the interferon polypeptides have identical amino acids. By introducing appropriate gaps, better homology could be achieved, particularly in the region of the

signal sequence; in the present comparison this has not been done.

To plot the degree of homology between the F-IF and Le-IF as a function of the map distance, the sequence was subdivided into segments of 8 amino acids (or 24 nucleotides), each overlapping by 4 amino acids (or 12 nucleotides) with the neighbouring segments, and the per cent coincidence of amino acids (and nucleotides) for each segment was determined (see ref. 23). As seen in Fig. 2, amino acid sequences show three domains of homology. The first one, with the least degree of homology, corresponds to the putative signal sequence, which is rich in hydrophobic residues and has 4 identical amino acid positions out of 21; the second domain, between amino acids 28 and 80 (counted on the Le-IF sequence), has 21 identical residues out of 51 (41% homology), and the third, between positions 115 and 151 (Le-IF sequence), has 19 out of 35 identical residues (54%). The longest stretches of contiguous conserved amino acids are Gln-Phe-Gln-Lys (positions 47-50 of Le-IF and 49-52 of F-IF) and Cys-Ala-Trp (positions 139-141 and 141-143, respectively); the latter sequence is notable because it comprises Cys and Trp, which are preferentially conserved in related proteins²⁴. Table 1 shows that conservation was highest between the interferon polypeptides (not considering the signal sequences) for Trp, Phe, Arg, Cys and Tyr residues, in agreement with the general experience that the amino acids most likely to be conserved between related proteins are Trp>Cys>Tyr>Arg>Phe. His (ref. 24). Even where amino acids are conserved, the codons show one or more nucleotide changes in half the instances. The codons of three out of seven conserved Leu residues are unrelated, as are two of four codons pertaining to conserved Ser residues. This suggests that there is a strong selective pressure favouring the conservation of several amino acids. It is quite likely that at least some of the conserved amino acids are essential for a function common to Le-IF and F-IF, perhaps the induction of the virus-resistant state in the target cell. These findings may provide guidelines for the tailoring of modified²⁵, possibly shorter polypeptides possessing certain activities of interferon.

Comparison of the nucleotide sequences of Le-IF and F-IF

The nucleic acid sequences show an average homology of 43% in the domain of the signal sequence and of 45% in the interferon polypeptide sequence. On a random basis, about

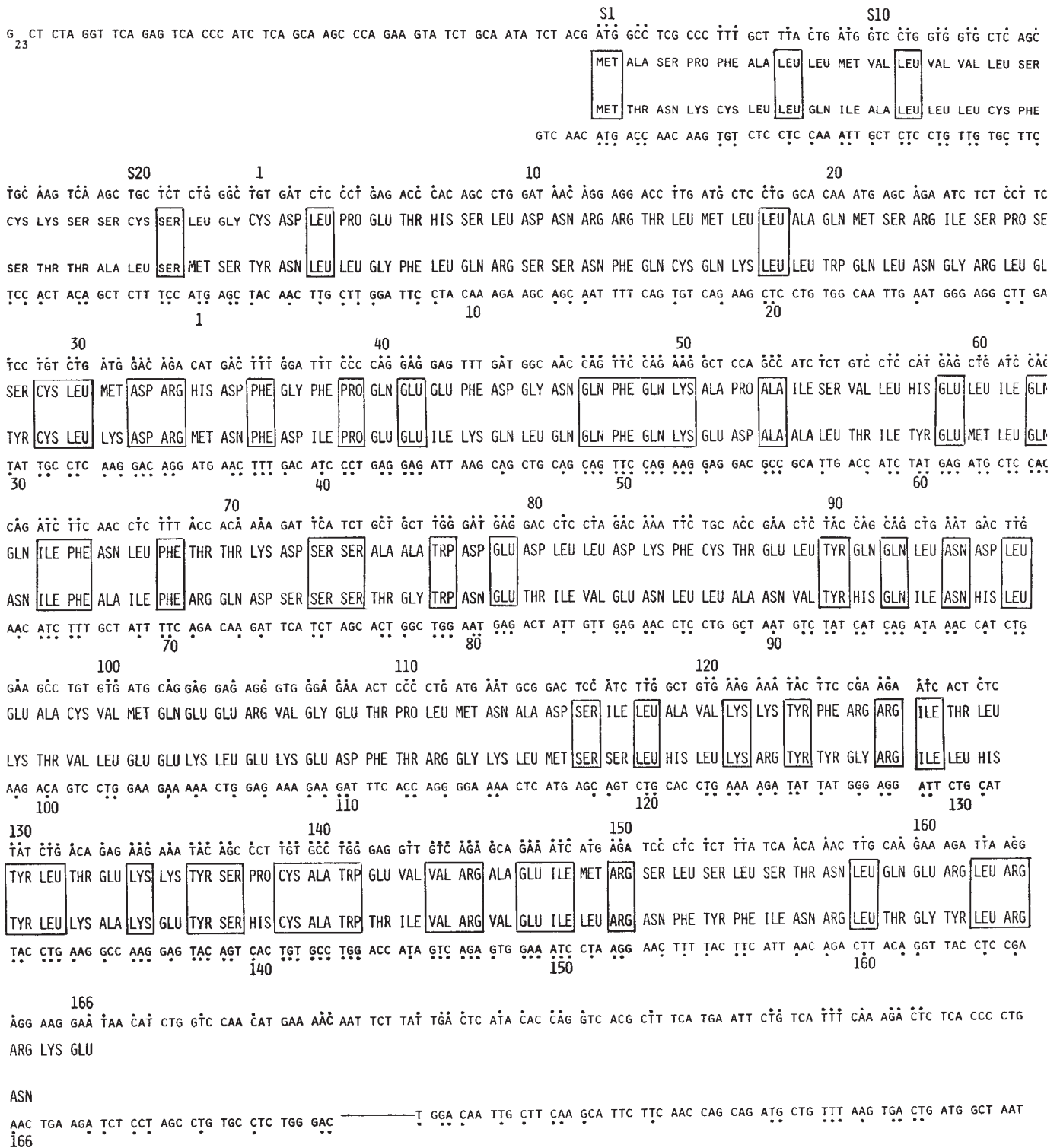


Fig. 1 Comparison of the nucleotide sequences of human leukocyte interferon I (Lc-IF I) and human fibroblast interferon cDNA and of the derived amino acid sequences. The sequences are from Mantei *et al.*²⁰ and T.T. *et al.*²². They were aligned to give maximal homology without introducing gaps in the coding sequence. Identical amino acids are framed, identical nucleotides are marked by a dot. S1-S23 indicate the amino acids of the putative signal sequence and 1-166 the amino acids of the interferon polypeptides.

25% of the nucleotide positions should coincide. Within the interferon coding sequence, the nucleotide homologies are more evenly distributed than the amino acid homologies. However, one may distinguish, albeit to a less pronounced degree, the same three blocks of similarity noted for the amino acids. The longest region without mismatches extends for 13 nucleotides (compare 47th to 51st codon of Le-IF with 49th to 53rd codon of F-IF). There are, in addition, sequences of 17, 18 and 20 nucleotides with 3, 3 and 4 mismatches, respectively. The heteropolymeric 3'-terminal noncoding region of Le-IF cDNA has 242 nucleotides, and is longer by 39 residues than its counterpart in F-IF cDNA. In aligning the two sequences, four gaps were introduced to maximize homology, as described by van Ooyen *et al.*²³. In this way, several segments were matched with 29–41% homology. The introduction of gaps in the alignment may be justified in view of the arguments presented previously, that intervening sequences and noncoding regions of reduplicated genes diverge as a consequence of block insertions and/or deletions in the course of evolution^{23,26}. It is unlikely that the extent of homology between Le-IF and F-IF cDNA would allow significant cross-hybridization between the two species.

A common ancestral gene for Le-IF and F-IF

On the basis of our findings, there is no doubt that Le-IF and F-IF genes are derived from a common ancestral sequence. When did the separation of these genes occur? Human α - and β -globin show 57% amino acid mismatches, and human β -globin and myoglobin, as well as α -globin and myoglobin, 76% mismatches. If the rate of divergence of interferons and globins is comparable (however, this is quite uncertain, see ref. 24, p. 50, for proteins showing both higher and lower rates), the separation of interferon genes occurred after that of myoglobin and haemoglobins but before that of α - and β -globins, that is between 500 and 1,000 Myr ago²⁴, which is about the time vertebrates arose²⁷. This would mean that both types of interferon gene should occur in all vertebrates, unless one and/or the other was lost by deletion. Indeed, as shown by the sequencing of 13–24 amino-terminal residues^{16,17,28}, mouse interferons A and B show significant homology with human fibroblast interferon, and mouse interferon C with human

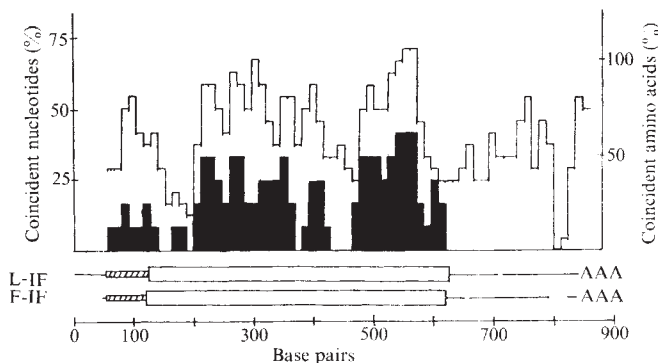


Fig. 2 Similarity of the nucleotide and amino acid sequences of human leukocyte interferon I and fibroblast interferon. The sequences shown in Fig. 1 were subdivided into segments of 8 amino acids or 24 nucleotides, each overlapping by 4 and 12 residues, respectively, with the neighbouring segments. The percentage of coincident residues was plotted as a function of map position. Open vertical blocks, nucleotides; filled vertical blocks, amino acids. L-IF, leukocyte interferon cDNA; F-IF, fibroblast interferon cDNA; lines, noncoding sequences; hatched bars, putative signal peptide; open bars, interferon polypeptide.

lymphoblastoid interferon, whereas the mouse species A and B on the one hand, and the species C on the other show no significant homology within the short segment sequenced. Thus, at least in the mouse, representatives of both interferon families exist. It will be of interest to determine the evolutionary relationship of these to the third type of interferon, immune or γ -interferon.

After submission of this article, we learnt that Derynck *et al.* had cloned and sequenced fibroblast interferon (see accompanying article²⁹), confirming the deduced amino acid sequence of T.T. *et al.*²².

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Table 1 Conservation of amino acids in leukocyte and fibroblast interferon

	F-IF	Le-IF	Conserved amino acids	No. of changes in codon of conserved amino acids			
				0	1	2	3
Leu	25	22	8	1	4	3	
Cys	3	5	2	1	1		
Asn	12	6	1	1			
Arg	11	12	5	1	3	1	
Phe	9	8	4	2	2		
Pro	1	6	1		1		
Gln	11	10	3	3			
Lys	11	8	3	2	1		
Ala	6	10	2	2			
Glu	13	15	4	4			
Ile	11	7	3	2	1		
Ser	9	13	4		2	1	1
Trp	3	2	2	2			
Tyr	10	4	4	1	3		
Val	5	6	1	1			
Asp	5	11	1	1			
Thr	6	9	0				
Gly	6	3	0				
Met	4	6	0				
His	5	3	0				
Total	166	166	48	24	18	5	1

The data are from T.T. *et al.*²² and Mantei *et al.*²⁰.

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Sequence of retrovirus provirus resembles that of bacterial transposable elements

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The nucleotide sequences of the terminal regions of an infectious integrated retrovirus cloned in the modified λ phage cloning vector Charon 4A have been elucidated. There is a 569-base pair direct repeat at both ends of the viral DNA. The cell-virus junctions at each end consist of a 5-base pair direct repeat of cell DNA next to a 3-base pair inverted repeat of viral DNA. This structure resembles that of a transposable element and is consistent with the provirus hypothesis that retroviruses evolved from the cell genome.

RETROVIRUSES are a family of RNA viruses that infect animals and replicate through a DNA intermediate, the provirus¹. The provirus hypothesis suggests that retroviruses evolved from the cell genome, in particular from a portion of the cell genome involved in normal transfer of genetic information in and between cells².

The recent availability of recombinant DNA techniques for cloning eukaryotic genes and of rapid DNA sequencing techniques has enabled us to isolate several infectious proviruses of spleen necrosis virus and their surrounding cellular sequences³ and to sequence several hundred bases including the cell-virus junctions of one of these proviruses. (Spleen necrosis virus is an avian retrovirus that has genus-specific relationships to mammalian type C retroviruses⁴⁻⁶.) The regions sequenced include the two cell-virus junctions, the complete terminal repeats, the positions of origin and terminus of viral RNA, and a binding site for a putative primer tRNA. In particular, the cell-virus junction sequences seem to have a

5-base pair direct repeat of cellular DNA next to an inverted repeat of viral DNA. This structure resembles that of bacterial transposable elements⁷⁻¹² and is consistent with the provirus hypothesis that retroviruses have evolved from part of the normal cell genome.

Sequencing of terminal regions of an integrated spleen necrosis virus

14-44 is a recombinant clone of the modified phage λ cloning vector, Charon 4A, containing chicken DNA with an infectious provirus of spleen necrosis virus (SNV)³. 14-44 was selected from phage packaged with Charon 4A arms ligated to *Eco*RI-digested DNA isolated from chicken cells 4 days after infection (acute infection). Restriction endonuclease cleavage sites in 14-44 have been mapped previously, as indicated in Figs 1 (top) and 2a (see ref. 3). In particular, the virus-cell junctions were located just left of the 5' *Sac*I site, and approximately 600 base pairs right of the 3' *Sac*I site and 200 base pairs left of the 3' *Eco*RI site by comparison with restriction enzyme digests of 10 cloned proviruses and of unintegrated viral DNA, and also by electron microscopic heteroduplexing³.

Subclones containing the junction regions were isolated as described in Fig. 1 legend. Fine structure restriction enzyme maps (Fig. 2b) were prepared for the subclones containing each end of the provirus using the method of Smith and Birnstiel¹³ with labelling of the *Sal*I end or the *Eco*RI end, respectively. Starting from the left end of both subclones, after

Fig. 1 Construction of subclones of 14-44 DNA in pBR322. DNA (100 μ g) of clone 14-44 was digested with *Hind*III and *Eco*RI, and the fragments separated by gel electrophoresis in 1% agarose. The DNA bands of 14-44 were visualized with the aid of ethidium bromide, were cut out, and the agarose removed by soaking the crushed gel in 1 M NaCl, 1 mM EDTA, 0.1% SDS, 20 mM Tris-HCl (pH 8.0) at 37°C for 20 h followed by ethanol precipitation. Each DNA fragment was then separately ligated with T4 DNA ligase at 4°C for 20 h to the large fragment of *Eco*RI-*Hind*III-digested pBR322 DNA. Calcium chloride-treated competent *Escherichia coli* cells were transformed with the ligated DNA and the cells plated on medium containing 70 μ g each of tetracycline and ampicillin. The colonies which appeared were transferred to nitrocellulose filters for colony hybridization⁴⁹. The filter was treated with 0.1 M NaOH for 1 min, neutralized with 1 M Tris-HCl (pH 7.2), 0.6 M NaCl, and dried at 80°C under vacuum to immobilize the DNA on it. The filter was hybridized with ³²P-labelled SNV cDNA (specific activity about 10⁸ c.p.m. per μ g) at 63°C for 20 h and washed to remove unhybridized cDNA. Autoradiographs were made after drying. The subcloned DNA which carried the 5' half *Eco*RI-*Hind*III fragment of 14-44 DNA was further digested with *Sal*I and self-ligated with T4 DNA ligase as described above. Calcium-treated competent *E. coli* cells were transformed, and cells were plated on Petri dishes containing 50 μ g ampicillin. An aliquot of each colony which appeared was transferred to agar-medium containing 50 μ g tetracycline, and plates were kept at 37°C for 24 h. Colonies which could not grow on the medium containing tetracycline contained the 5' *Eco*RI-*Sal*I DNA fragment of 14-44. Plasmid DNA was purified from chloramphenicol-treated bacteria which contained the subcloned plasmids as follows: The bacteria which contained the plasmids were grown to late log phase. Chloramphenicol was added to a final concentration of 20 μ g per ml of culture medium. Incubation was continued another 5 h. Cells were collected by centrifugation and lysed with EDTA, lysozyme and Brij 58 (ref. 50). Cellular DNA was pelleted by centrifugation at 27,000 r.p.m. for 60 min. The supernatant was taken, CsCl added, and the refractive index adjusted to 1.395. Ethidium bromide was also added to a final concentration of 50 μ g per ml of solution. The lysates were centrifuged at 35,000 r.p.m. for 72 h using a Beckman 50 Ti rotor. The lower DNA band was collected and centrifuged at the same speed for 48 h. The DNA band was taken out, the ethidium bromide was removed by extraction with isobutyl alcohol, and the DNA was dialysed and ethanol precipitated.

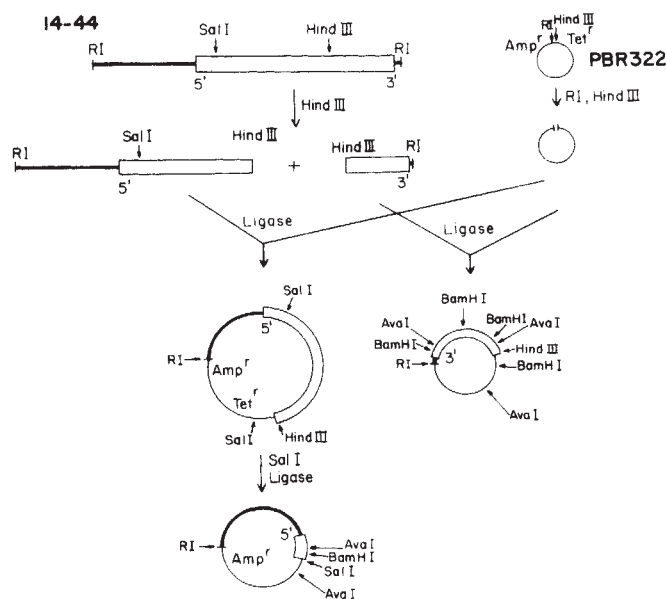
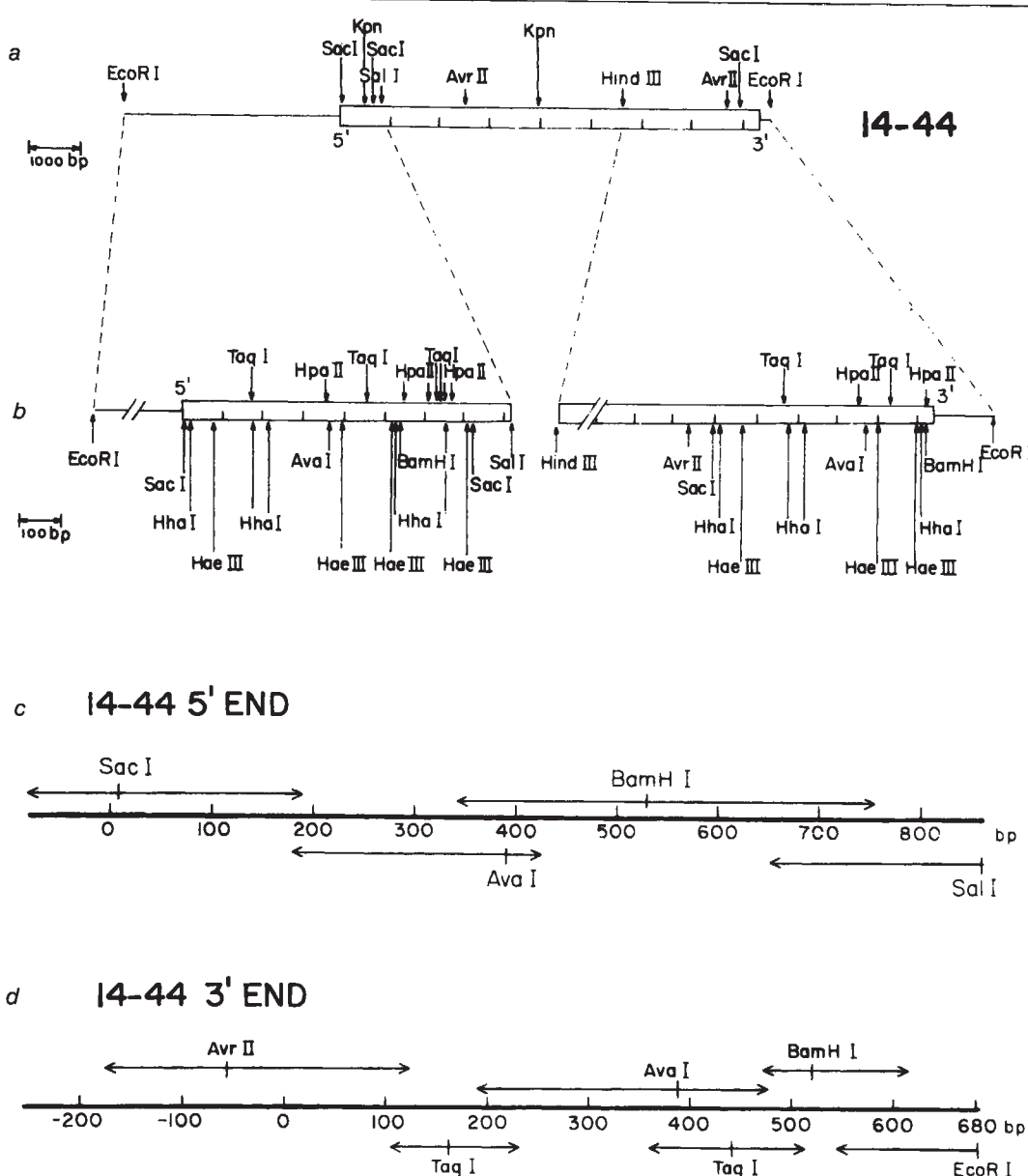


Fig. 2 Structure of recombinant clone 14-44 and subclones containing 5' and 3' ends of SNV. **a**, Restriction map of the inserted DNA of clone 14-44, containing an infectious provirus of SNV³. 5' and 3' are defined in terms of the ends of viral RNA¹⁴. The formation of subclones containing the 5' and 3' junction sequences is described in Fig. 1 legend. Mapping of the cleavage sites of restriction endonucleases in the subclones (**b**) was done as follows: The purified subcloned DNAs in pBR322 were digested with *SacI* or *EcoRI*. After removal of phosphate residues from the 5' end of the fragments with alkaline phosphatase, the 5' ends were labelled using polynucleotide kinase in the presence of [γ -³²P]ATP. Labelled DNAs were further digested with *EcoRI* or *HindIII*, respectively, and were separated by agarose gel electrophoresis. The DNAs were extracted from the agarose as described in Fig. 1 legend and purified by ethanol precipitation. Labelled DNAs were partially digested with restriction endonuclease (2 min, 4 min, 8 min and 15 min incubation at 37 °C in 50 μ l of reaction mixture containing 1 μ g DNA and 1 unit restriction enzyme), and fragments were separated by gel electrophoresis in 1.5% agarose at 1.5 V cm⁻¹ for 8 h. Gels were dried and autoradiographs made. The molecular weight of each fragment was calculated from the extent of migration of labelled restriction fragments. End-labelled *HindIII* and *TaqI* fragments of pBR322 DNA were used as markers in the agarose gel electrophoresis. **c, d** The extent of sequence determined from restriction enzyme fragments labelled at their 5' ends with ³²P as described by Maxam and Gilbert²¹. Labelled termini are indicated by (—) and the direction and extent of sequencing with arrows. Fragments for sequencing were isolated using agarose or polyacrylamide gel electrophoresis. In each case, after 5' labelling, molecules were digested with another restriction enzyme as indicated below. (Numbering is from the beginning of the terminal repeat (see Fig. 4).) **3' end.** *EcoRI*: digested with *HindIII* and the 2.6-kilobase-pair fragment was isolated. *BamHI*: The 4-kilobase pair fragment was isolated and digested with *EcoRI* and the 160-base pair fragment was isolated. The 1.7-kilobase-pair fragment was isolated and digested with *AvaII* and the 570-base pair fragment was isolated. *AvaI*: The 3.6-kilobase-pair fragment was isolated and digested with *EcoRI* and the 300-base pair fragment isolated. The 2.5-kilobase pair fragment was isolated and digested with *AvaII* and the 420-base pair fragment was isolated. *AvrII*: digested with *EcoRI* and the 700-base pair and 6.7-kilobase pair fragments were isolated. *AvrII* to *EcoRI* 750-base pair fragment was digested with *TaqI*. The 260-base pair fragment was digested with *BamHI* and the 170-base pair fragment was isolated. Half the 250-base pair fragment was digested with *AvaI* and the 200-base pair fragment was isolated. The other half of the 250-base pair fragment was digested with *HhaI* and the 210-base-pair fragment was isolated. **5' end.** *SacI*: digested with *EcoRI* and the 6-kilobase pair fragment was isolated. *BamHI*: digested with *EcoRI* and both fragments were isolated. *AvaI*: digested with *EcoRI* and both fragments were isolated. *SacI*: digested with *EcoRI* and *AvaI* and the 4.2-kilobase-pair and 400-base-pair fragments were isolated.



the *AvrII* site present only in the subclone with the 3' end of SNV, the same cleavage sites are seen: *SacI*, *HhaI*, *HaeIII*, . . . up to the *HaeIII*, *HhaI*, *BamHI* and *HpaII* sites. Then the maps diverge. These data indicate that the terminal regions in the SNV provirus are repeated, as they are in the unintegrated SNV DNA¹⁴, and that the terminal repeats are near to or at the ends of the viral DNA, as defined by comparison with the restriction cleavage sites in 10 cloned proviruses and in unintegrated DNA and by electron microscopic heteroduplexing of several of the cloned proviruses³.

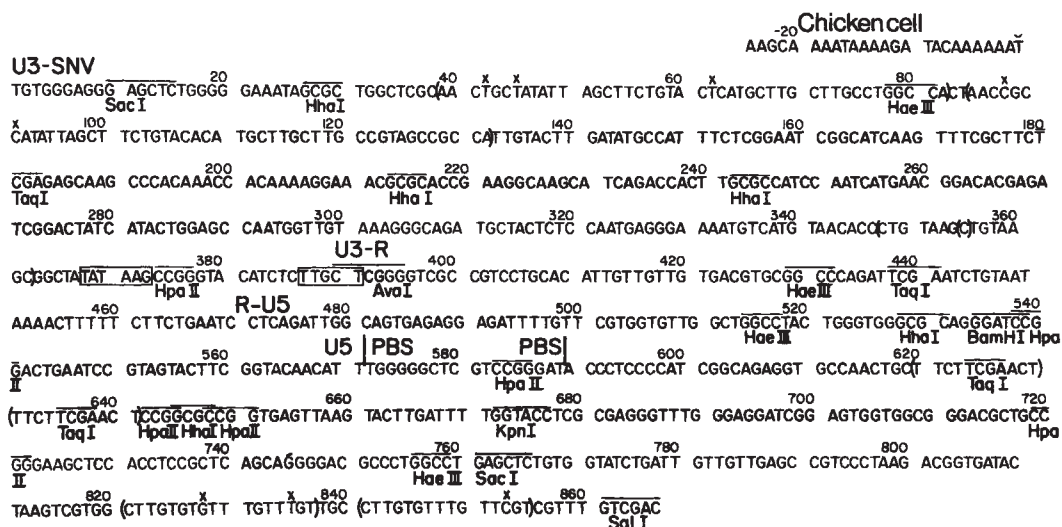
DNAs of the pBR322 subclones containing the 5' and the 3' cell-virus junctions were digested with different restriction endonucleases, and the fragments were isolated, labelled, and further digested by restriction enzymes as described in Fig. 2c, d. The fragments were then sequenced as illustrated in Fig. 3.

Nucleotide sequences of terminal regions of provirus in 14-44

The sequences of the 890 bases at the 5' end of SNV in 14-44 and the 850 bases at the 3' end of 14-44 are given in Fig. 4. These sequences contain all of the restriction endonuclease sites mapped at the ends of the virus DNA (Fig. 2b), as well as the *AluI* and *HinfI* restriction endonuclease sites which were also mapped in the plasmid DNA (data not shown).

Comparing the sequences of the 5' and 3' ends directly, one sees that a 569-base pair sequence from nucleotides 1 to 569 at the 5' end is the same as the nucleotide sequence from nucleotides 1 to 569 at the 3' end. The sequences to either side diverge. Therefore, the terminal repeat in viral DNA is 569 base pairs. This is similar in size to that of the terminal repeat

14-44 5' End



14-44 3' End

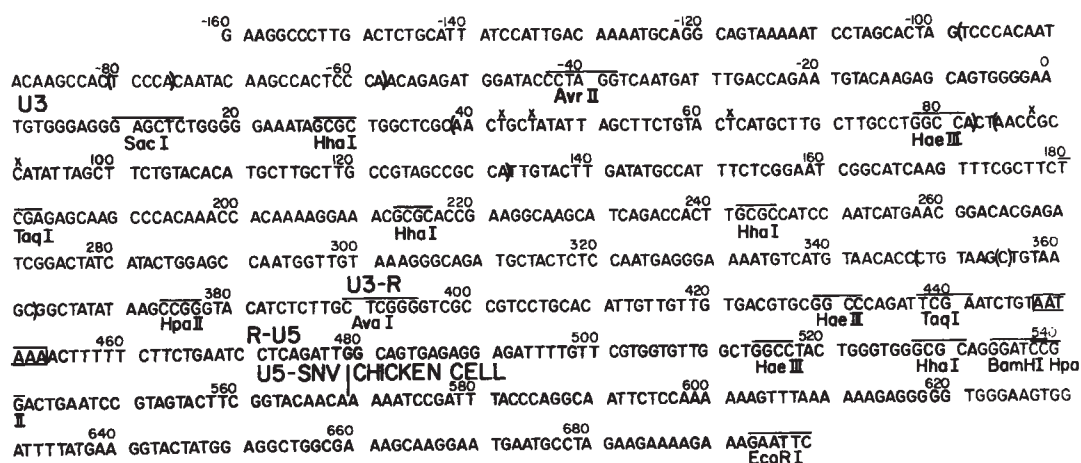


Fig. 5 Portions of sequencing gels with the ends of the terminal repeats of viral DNA. Portions of the sequencing gels used to secure the sequences shown in Figs 4 and 6 are presented. NT, nucleotides. 14-44 3' end NT -11 to 25 is from a gel labelled at the *Ava*I cleavage site; 14-44 5' end NT 4 to -17 is from a gel labelled at the *Sac*I cleavage site; and 14-44 5' and NT 564 to 584 and 14-44 3' end 548 to 574 are from gels labelled at

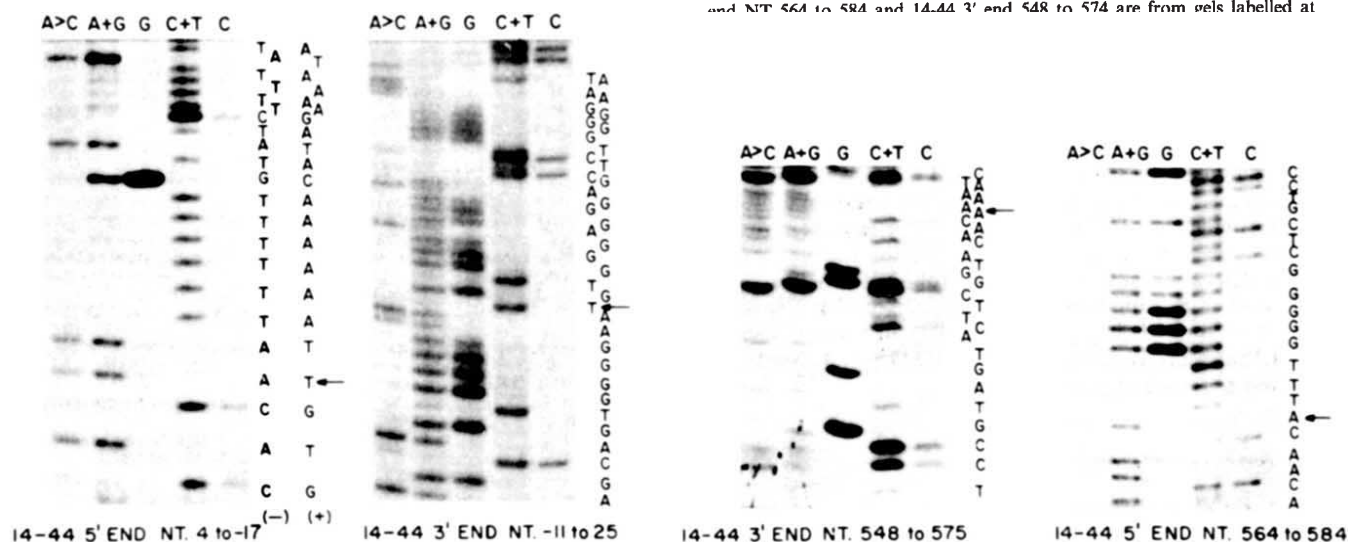
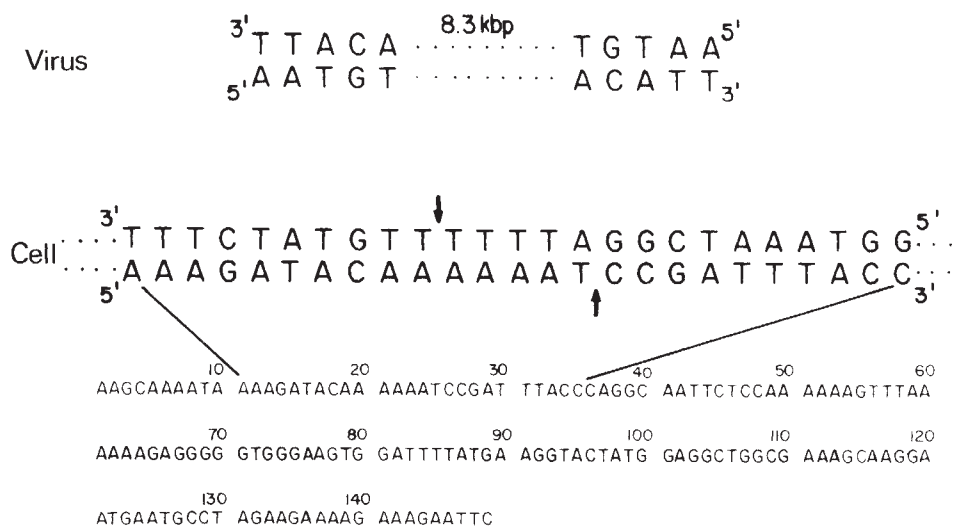


Fig. 6 Proposed sequences of viral and cell precursors to provirus. The viral genome is that of a linear un-integrated DNA with terminal repeats at both ends starting at the end of the primer binding site and the analogous nucleotide on the 5' (U3) end. Integration occurs two bases from each end in the centre of a 5-base pair inverted terminal repeat. The cell DNA is nucleotides -24 to 0 of 14-44 5' end (Fig. 4) and nucleotides 570 to 698 of 14-44 3' end (Fig. 4) with an overlap of five bases. Nucleotide 1 corresponds to nucleotide -24 of 14-44 5' end; and nucleotide 30 corresponds to nucleotide 579 of 14-44 3' end. Arrows in the cell DNA represent possible cleavage sites of a putative nuclease. Alternatively, a 5' overhang could be formed.



Transposable elements also end with inverted repeats, although usually they are over 20 base pairs long^{7-12, 35-42}.

The integration of viral DNA may require only one or two specific nucleases and a ligase. The presence of DNase and DNA ligase activities in purified virions of avian retroviruses has been reported⁴³⁻⁴⁵. These findings support the hypothesis that retrovirus virions contain all the proteins necessary for provirus synthesis⁴⁴.

The sequences in the provirus next to the 5' end and near the 3' end are G+C rich. This base composition may be significant.

The sequence of cell DNA around the site of insertion may be reconstructed (Fig. 6). It is very A+T rich (73% A+T; chicken DNA is 58% A+T) and has no stop codons in one reading frame. Further sequencing of proviral clones will be required to determine if these characteristics or the exact sequence at the integration site have any specificity or if they are correlated with proviral infectivity^{14, 46}. The sites of insertion of Tn3 seem to be A+T rich⁴⁷.

The resemblance of the provirus to transposable elements indicates that retroviruses may have evolved from such elements. Evolution might possibly have involved the appearance of start and stop sequences for RNA synthesis in an insertion element⁴⁸; appearance of a sequence that could bind a tRNA in the insertion element proximal to the site of the start sequence for RNA; the presence of a DNA polymerase in cells that could use this tRNA as a primer and the RNA as a template; formation of a transposon by integration of two of these insertion sequences around the coding sequences for this polymerase; and addition of nucleotide sequences for the rest of the present virion proteins. As the transposon transposed through an RNA intermediate, a long inverted repeat would not be needed to mark its end, as the end would also be the end of a molecule. Therefore, the size of the inverted terminal repeat would have decreased.

Since submission of this article, the same pattern of a 5-base pair repeat of cellular DNA, different in every case, next to the 3-base pair inverted repeat of viral DNA, the same in every case, has been found in five other clones of SNV proviruses.

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LETTERS

Far UV observations of PKS2155-304

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In BL Lac objects the absence of broad emission lines may be due either to a lack of ionizing continuum or to a lack of cool gas ($T \lesssim 10^4 \text{ K}$)¹. UV observations of BL Lac objects are interesting in that they provide a measurement of the continuum, and they include the wavelength range of Ly α , which is in principle the most favourable line for detection. We report here several spectra of the BL Lac object PKS2155-304 in the 1,150-3,200 Å band taken with the IUE when the object was in a bright phase. The UV flux connects smoothly with the optical and IR observations of the source in its brightest state and its extrapolation matches the soft X-ray flux, implying a change in spectral slope around 10^{15} Hz .

This object was discovered as a result of X-ray observations by HEAO I of a region of the sky where the source 2A2151-316 was reported by the Ariel 5 Catalogue². The precise positioning identified it with a 14 mag star-like object and with the radio source PKS2155-304 (refs 3-5). The observation in the optical range of a blue featureless continuum with polarization of $\sim 5\%$ (refs 6, 7) indicated its BL Lac nature. A weak emission feature subsequently detected by Charles *et al.*⁸ was confirmed by further observations by one of us (M.T.). If the feature is identified with the O III doublet ($\lambda\lambda 4,959, 5,007$) as proposed by Charles⁸, the corresponding redshift is 0.17, which makes this the most intrinsically luminous BL Lac object known thus far. In the past 40 years its visual magnitude has ranged from 12.8 to 14.2 (ref. 7). Recent optical observations show variability in flux and spectral shape on time scales of about one month. Variability in X rays

seems to be more rapid, on a day scale. No data on variability at radio and IR frequencies have yet been obtained.

IUE observations were performed using a blind offset technique with coordinates:

$$\alpha(1950) = 21 \text{ h } 35 \text{ min } 58 \text{ s } 0.33 \text{ s } \delta(1950) = -30^\circ 27' 53.7''$$

as measured on the glass copy of the ESO quick blue sky survey. Five spectra were taken with the short wavelength camera (SWP: range 1,100-2,000 Å) and three with the long wavelength camera (LWR: range 2,000-3,000 Å). The large aperture ($10 \times 20 \text{ arcs}$) and the low resolution mode were used for each observation. Observations are listed in Table 1: the data were reduced to absolute fluxes using the calibration curves of Bohlin *et al.*⁹. The spectrum SWP 6855 is heavily saturated in extended wavelength regions; the other spectra taken each day, summed and combined to cover the entire wavelength interval, are shown in Fig. 1 together with the portion of the long-exposed spectrum where saturation is incomplete. No correction has been applied for reddening. The absence of a broad absorption feature around 2,200 Å infers an upper limit $E(B-V) \sim 0.02$ (ref. 10).

In the region around 1,900 Å, where the range of the two cameras joins, a mismatch is apparent, probably due to inadequacies of the LWR calibration. The spectra are affected by microphonic noise, which makes identification of the weak features difficult. However, several dips visible in all individual spectra can be tentatively identified with galactic absorptions (see Table 2). In particular C IV ($\lambda 1,550$) and Al II ($\lambda 1,670$) show up clearly in the region of the long exposed spectrum (see inset in Fig. 1). The absorptions compare well with those observed by Savage and de Boer¹¹ and by Ulrich *et al.*¹², except for Mg II at 2,800 Å, which is not detectable in our spectra.

The emission expected to be strongest is Ly α , which, for a redshift of 0.17, should be at 1,420 Å. A small feature is present at this wavelength in the long exposed spectrum, which is not completely saturated in this region. In the short exposures, however, the feature is absent, and an upper limit of about 1 Å can be placed on its equivalent width, assuming a FWHM of

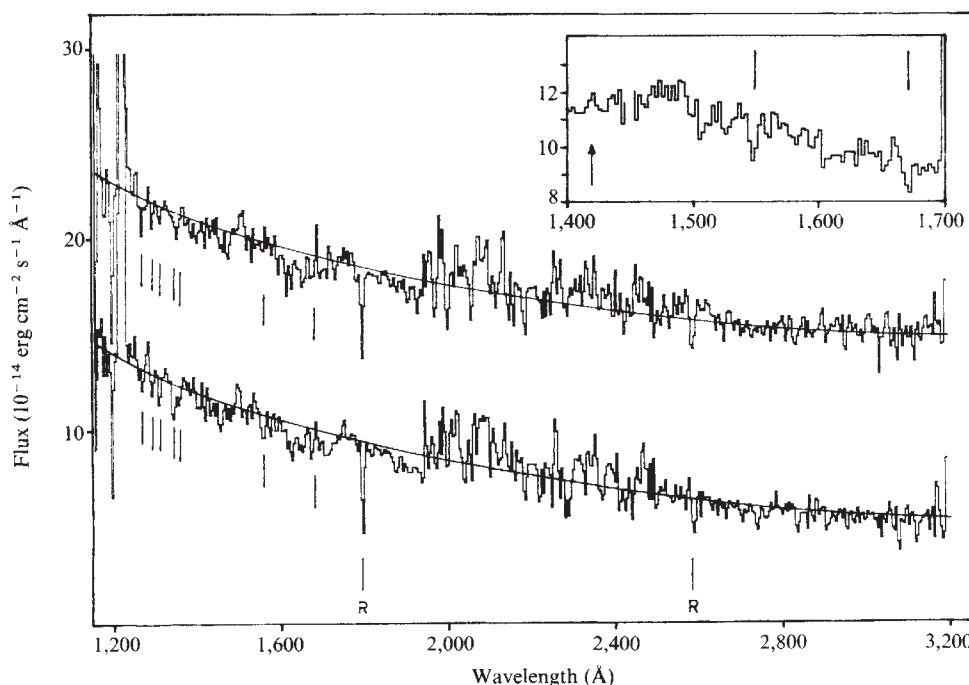


Fig. 1 Combined spectra of 13 and 14 October 1979 shown with a λ^{-1} fit for the continuum. The upper spectrum (14 October) has been displaced by $10^{-13} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ Å}^{-1}$. The inset shows the overexposed spectrum of 13 October in the region where saturation is not complete. Vertical lines correspond to the absorption features reported in Table 2. The position of the expected Ly α emission is indicated by an arrow.

Table 1 Integral fluxes in units of $10^{-12} \text{ erg cm}^{-2} \text{ s}^{-1}$

Image no.	SWP 6855	LWR 5828	SWP 6856	LWR 5829	SWP 6857	SWP 6867	LWR 5839	SWP 6868
Epoch October 1979 (UT)	13.700	13.797	13.823	13.846	13.872	14.642	14.674	14.706
Exposure time (min)	240	30	40	21.5	48	48	30	48
$F_{1,250-1,450}$	Heavily saturated		24.9		24.5	22.5		22.1
$F_{1,450-1,650}$	21.5		20.9		21.8	18.9		18.8
$F_{1,650-1,850}$	Heavily saturated		18.3		18.6	17.1		16.8
$F_{2,000-2,220}$		19.0		19.6			16.3	
$F_{2,200-2,400}$		15.8		16.8			14.8	
$F_{2,400-2,600}$		13.9		14.7			13.1	
$F_{2,600-2,800}$		12.2		12.8			11.5	
$F_{2,800-3,000}$		11.9		12.1			10.6	

10 Å. Given this upper limit other emissions should be undetectable. Note, however, that the two features at 1,390 and 1,495 Å which are present in all the spectra, if real, may correspond to C I (λ 1,164) and (λ 1,277). The second of these lines has been observed in emission in M31 (ref. 13).

If, as indicated by the overall spectrum, the continuum can be extrapolated beyond the Lyman limit the absence of strong emission lines cannot be ascribed to a deficiency of exciting UV photons, but rather to physical conditions of the gas surrounding the central source different from those of Seyferts and QSOs. This is also true for the two other BL Lac objects observed in the UV, Markarian 421 and 501 (refs. 14, 15).

Apart from the region 2,000–2,200 Å, a good fit is obtained with a power law of spectral index -1 , with $F_{\lambda} = 1.70 \times 10^{-10} \lambda^{-1}$ and $1.55 \times 10^{-10} \lambda^{-1} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ Å}^{-1}$ for 13 and 14 October, respectively. Taking into account the possible reddening correction, the spectral index would change at most up to -1.1 . According to the performance characteristics of IUE⁹ the flux variation between 13 and 14 October over the entire wavelength range is significant, and appears, in fact, between each of the spectra taken on different days (see Table 1), while spectra taken within few hours do not show significant variations.

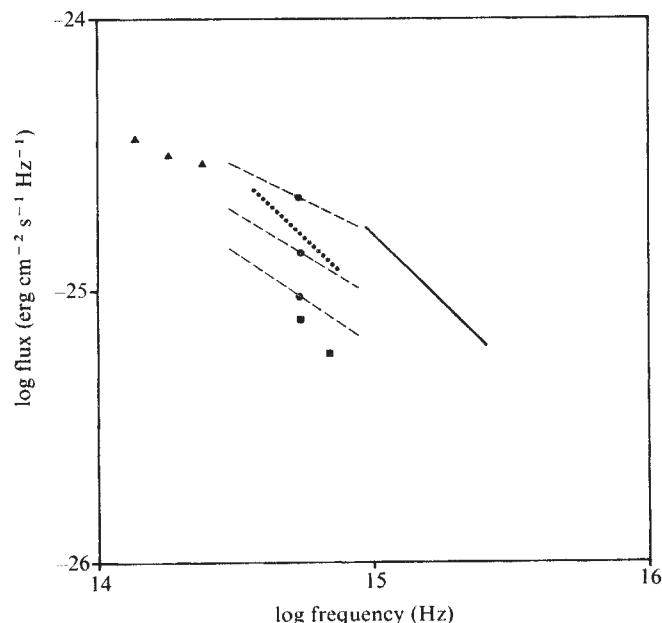


Fig. 2 Variability of PKS2155–304. —, 14 October 1979, from this paper; ---●---, 23 October 1978 (lower), 2 November 1978 (medium), 24 December 1978 (upper) from ref. 19; 2–3 December 1978, from ref. 18; ■, 24–27 October 1978 from ref. 8; ▲, 24 December 1978, from ref. 17.

The UV spectrum is compared with the optical data in Fig. 2, which summarizes the available information on the variability in this band. At the epoch of the UV observations the B magnitude, as given by the onboard optical monitor (FES), was between 12.8 and 13.1. The spectral index in the optical band, measured by Greenstein *et al.*¹⁷ when the source was at the same intensity level, was $\alpha = 0.5 \pm 0.1$, indicating a significant difference with respect to that in the UV band. This conclusion is, however, tentative, because the spectral index in the optical is variable and the observations were not simultaneous: for example a spectral index in the optical, $\alpha = 0.95 \pm 0.15$, is reported by Griffiths *et al.*⁷, on 2 December 1979, when the intensity (as derived from Fig. 4 of ref. 7) was lower by 40%.

For the other two BL Lac objects observed in UV, MK421 and MK501 (refs 14, 15) the slope of the continuum is 1.2 and 0.57 respectively, in agreement with the extrapolation of their optical spectra. Comparison of the optical and UV data with radio, IR and X-ray observations for these objects, indicates a spectral 'break' at $\nu_B \approx 10^{13} \text{ Hz}$ and $\nu_B \approx 10^{16} \text{ Hz}$ in the two cases^{13, 14}.

The overall spectrum of PKS2155–304 is shown in Fig. 3 which only includes optical data for the brightest state. The X-ray data, taken 40 days before the optical ones, lie on the extrapolation of the UV continuum thus supporting the spectral steepening around $\nu_B = 10^{15} \text{ Hz}$. The slope variation in the optical band, suggests that ν_B may be variable with a higher value in the brightest state.

For the three BL Lac objects observed in the UV in the radio to soft X-ray spectrum seems to be a two component spectrum with a rather abrupt break at a frequency ν_B , which for different objects varies between 10^{13} and 10^{16} Hz .

An interpretation in terms of radiative losses of relativistic electrons yields a relationship between the magnetic field and the lifetime t_c of the particles radiating at ν_B :

$$\nu_B = 3.5 \times 10^{23} \frac{B}{(B^2 + 8\pi W_{ph})^2 t_c^2} \text{ Hz}$$

where W_{ph} is the photon energy density in erg cm^{-3} . For PKS2155–304 the optical variability time scale, $t_v \sim 1$ month,

Table 2 Absorption lines of galactic origin

Observed wavelength (Å)	Proposed identification
1,261	Si II (1,263)
1,284	C I (1,281)
1,304	O I (1,305) + Si II (1,304)
1,335–1,350	C II (1,336) + not identified
1,550	C IV (1,584, 1,551)
1,669	Al II (1,671)

and the known distance allow a direct evaluation of $W_{ph} \approx 1 \text{ erg cm}^{-3}$. The maximum lifetime is obtained for $B^2/8\pi \approx W_{ph}$ ($B \approx 5 \text{ G}$) and $v_B \sim 10^{15} \text{ Hz}$ implies that $t_e \approx 10^3 \text{ s}$. As $t_e \ll t_v$ a continuous acceleration mechanism is required with a time scale $\sim t_e$. The synchrotron self absorption frequency for these parameters is 10^{11} Hz , implying that the radio emission comes from a larger region.

Another interpretation of the spectral steepening is that the flat part is due to synchrotron radiation from a mildly relativistic gas ($T \approx 10^{10} \text{ K}$) in a high magnetic field $B \sim 10^6 \text{ G}$, while the steeper part is due to multiple Compton scattering of the synchrotron photons in the hot gas²⁰.

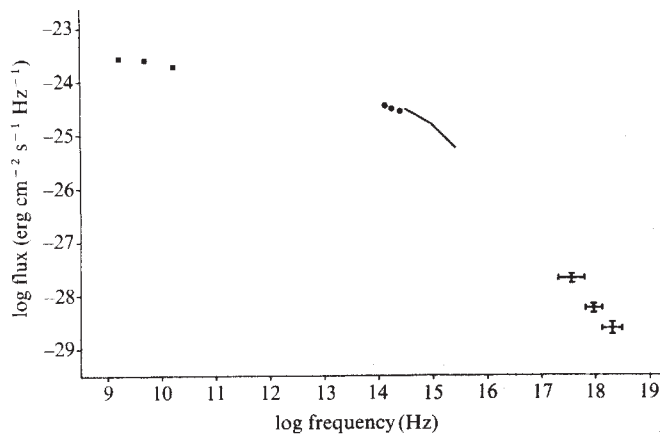


Fig. 3 Overall spectrum of PKS2155-304 near maximum brightness. Radio data are from ref. 16, IR data from ref. 17, optical data (24 December 1978) from ref. 19, UV data (13-14 October 1979) from this paper, X-ray data (8 November 1978) from ref. 5.

The main difference between the two hypotheses is that in the second the mean electron energy is lower and the dimension of the source is smaller. Discriminating features would therefore be variability on short time scale and a cutoff in the spectrum in the MeV region due to the low effective electron energy and high opacity.

Our observations of PKS2155-304 show that as in MK421 and MK501 the absence of emission lines is not due to the absence of a UV continuum. The break between the optical and UV bands is an important constraint on the emission mechanism. Further observations would be useful for studying the variability of this feature.

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Electromagnetic scattering lifetimes for dust in Jupiter's ring

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Dust particles of $4 \mu\text{m}$ radius which are reported to lie in a ring at 1.8 jovian radii around Jupiter may be charged to -10 V , but any larger charge would lead to non-circular orbits, due to interactions with Jupiter's dipole magnetic field. Such dust will be scattered randomly in eccentricity and inclination by variations in Jupiter's magnetic field strength as seen by the dust particles. We report here that this scattering will effectively dissipate the ring in a time scale less than 100 yr; and that the ring must, therefore, be constantly replenished, as suggested by Burns¹, or else it is a short-lived phenomenon.

The discovery of a ring of micrometre-sized particles orbiting Jupiter at a distance of 1.8 jovian radii² has raised speculations as to the lifetime of such particles against perturbing forces. Burns¹ pointed out that such particles will be subject to a Poynting-Robertson drag (with a lifetime of 10^5 yr) and will be eroded by sputtering with magnetospheric particles over a time scale of 10^2 - 10^5 yr . He suggests such particles originate from the regolith of one or more small (1-10 km) satellites; they may be fragmented and eroded by sputtering, electrostatic bursting, or meteoric collisions and self-collisions to their characteristic size of $4 \mu\text{m}$ (refs 3, 4).

Such particles existing in the circum-jovian plasma will certainly have a surface charge and interact with the jovian magnetic field, as pointed out by Hill and Mendis⁴. In the case of Jupiter, the field in the region of the rings is still essentially a dipole⁵ of 4.25 G and the plasma around Jupiter at this radius can be considered co-rotating with Jupiter at $1.74 \times 10^{-4} \text{ s}^{-1}$ (ref. 6). This co-rotation leads to an electric field of $\mathbf{E}_c = -(\boldsymbol{\omega} \times \mathbf{r}) \times \mathbf{B}$ where $\boldsymbol{\omega}$ and $\mathbf{B}$ are Jupiter's spin frequency and magnetic field vectors, and $\mathbf{r}$ the radius vector from the spin axis. In addition, particles orbiting with a velocity $\mathbf{v}$ will also see an electric field equal to $\mathbf{E}_0 = (\mathbf{v} \times \mathbf{B})$. Defining a term $\gamma = qr_0^3/mc$, where q is the charge on particles of mass m , and given B_0 , the strength of the magnetic field at radius r_0 , we can calculate the equations of motion in r and θ directions:

$$\ddot{r} - r\dot{\theta}^2 = -\frac{\mu}{r^2} + \frac{\omega\gamma B_0}{r^2} - \frac{\dot{\theta}\gamma B_0}{r^2} \quad (1)$$

$$\frac{1}{r} \frac{d}{dt} (r^2 \dot{\theta}) = \frac{\dot{r}\gamma B_0}{r^3} \quad (2)$$

where μ is the keplerian constant (here equal to the gravitational constant G times the mass of Jupiter). Equation (2) can be integrated directly to yield

$$r^2 \dot{\theta} = -\gamma B_0 / r + h$$

where h , the constant of integration, is the angular momentum of the orbiting particle per unit mass. From this, one can solve for $\dot{\theta}$ and substitute directly into equation (1) to yield

$$\ddot{r} = -\frac{\mu}{r^2} + \frac{h^2}{r^3} - \frac{3\gamma B_0 h}{r^4} + \frac{\omega\gamma B_0}{r^2} + \frac{2(\gamma B_0)^2}{r^5} \quad (3)$$

In a circular orbit with $\gamma=0$, one sees the expected result that $h^2 = \mu r$; magnetic field effects will become important only when the last three terms are of comparable magnitude to the first two.

For spherical particles it can be shown that $\gamma = 9.7 \times 10^{15} V/b^2 \rho$ where V is the electrostatic voltage on the dust particles, and ρ and b the dust mass density and radius. For ring particles with $\rho = 2 \text{ g cm}^{-3}$ and $b = 4 \mu\text{m}$, equation (3) can be integrated numerically to find the range of r traversed by ring particles.

The results of such an integration show that particles charged to -10 V will produce a ring as wide as the observed bright outer ring ($0.05 R_J$). A charge of -100 V would spread particle orbits out to a much greater extent than is observed. One can thus conclude that -10 V is an upper limit on the charge.

Theoretical estimates for the charge on the dust in Jupiter's plasma depend on a better knowledge of the typical plasma energies than are currently available. Intriligator and Wolfe⁷ suggested that a 100 eV plasma may exist close to Jupiter, which would produce dust voltages of -200 V if the charge on the dust were in thermal equilibrium with the plasma. Hill and Mendis⁴ also calculated that dust $1 \mu\text{m}$ or larger in Jupiter's magnetosphere should bear potentials of many hundreds of volts. However, as we have just seen, such high voltages for ring particles are incompatible with the observed width of the ring. More recently, work by Johnson *et al.*⁸ using Voyager data estimate the voltage on dust inside Io's orbit to be -10 V , which is compatible with the ring width.

This dust will travel in slightly eccentric orbits, but these orbits will no longer be closed. The smaller the dust, the more eccentric the orbit and the wider the range of r traversed. Because γ varies as b^2 , particles of $1 \mu\text{m}$ will be an order of magnitude more strongly affected than the $4 \mu\text{m}$ ring particles, and will quickly spread away from the rings, while $10 \mu\text{m}$ particles will be virtually unaffected.

The effect of this extra force is outward, as the rings are within the synchronous orbit point; thus if the ring dust is produced by the eroding of larger particles in originally circular orbits, these particles will immediately spread away from Jupiter in orbits whose perijoves are at the orbit of the source material. This argues against the observed small satellite at the outer edge of the rings being the direct source of the ring particles; the sources ought to be at the inner edge of the ring, if the ring width is determined by electromagnetic forces.

In addition to the extra force terms which the magnetic field adds to the equation of motion of the dust particles, fluctuations within the field can cause a scattering of particle orbital elements in a manner similar to that outlined for interplanetary dust⁹. For brevity, let us consider the case where the eccentricity $e=0$ and i is small (here, i will be the tilt of the dipole, or 10°).

The perturbing accelerations R , N and T in the directions radial, normal to the orbit plane, and in the direction of the orbit arise from the cross product of a B field whose strength varies as a dipole but whose direction is varying randomly in all coordinates, crossed with the orbital velocity of the dust and the co-rotating velocity of the plasma.

For $e=0$, the change in semimajor axis, a , depends only on T ; but T must be zero, as all accelerations must be normal to the particle or plasma velocity vector. Thus no change in a is expected.

The acceleration $R = \gamma[v_\theta B_\phi - v_\phi B_\theta] \approx \gamma[(h/a)(-\sin i \cos \eta B_{0\phi} - (\cos i - r\omega)B_{0\theta})]$. If $T=0$, the change in e depends entirely on R and can be written

$$\frac{de}{dt} = \gamma \sin \eta [\sin i \cos \eta B_{0\phi} + (\cos i - a^2 \omega \cos i/h) B_{0\theta}]$$

Following the procedure outlined by Consolmagno⁹, this equation is squared, averaged over one orbit, and integrated over time intervals which are long compared with the correlation time for changes in the magnetic field, arriving at

$$\langle \Delta e^2 \rangle = (\gamma/a^3)^2 [\sin^2 i P_{\phi\phi}/8 + (\cos^2 i + a^3 \omega^2/\mu - 2a\omega \cos i/(a\mu)^{1/2}) P_{\theta\theta}/2] \Delta t \quad (4)$$

where $P_{\phi\phi}$ and $P_{\theta\theta}$ are the power spectral densities of the magnetic fields.

Likewise the N component can be calculated, and a mean square change in inclination derived, to be

$$\langle \Delta i^2 \rangle = \gamma^2 \sin^2 i (1 + a^3 \omega^2 \cos^2 i/\mu - 2a\omega \cos i/(a\mu)^{1/2}) P_{\pi\pi} \Delta t / (8a^6) \quad (5)$$

Finally, we must derive values for P_{rr} , $P_{\phi\phi}$ and $P_{\theta\theta}$. In each case we will assume these are equal to some portion of the mean square dipole field strength times some correlation time.

The ϕ component arises only from fluctuations of the θ direction field. We can assume these fluctuations to be sinusoidal with an amplitude of 0.1 times the field strength (matching observed B_ϕ strengths¹⁰) and a period of roughly 10 min (matching observed fluctuations in the B field¹¹). Although the θ component field itself does not reverse, we can assume that fluctuations within the field will exist and produce a $P_{\theta\theta}$ component of the same magnitude as $P_{\phi\phi}$.

For $P_{\pi\pi}$ we note that the B_r field will appear to reverse itself as the particle passes above and below the magnetic equator. Furthermore this field is offset from the centre of Jupiter by $0.1 R_J$. The period of oscillations is roughly the synodic period of the particles and the rotating dipole ($\sim 25 \text{ h}$ for ring grains). Recall that the strength of the dipole falls as the cube of the radial distance; due to the offset dipole and the eccentricities produced by the non-keplerian forces discussed above, the grains will not see equal field strengths above and below the equator, so the average effect will not cancel out. The net effect will depend on the initial orientation of the dust and on variations in its eccentricity, both of which depend on the history of the dust which cannot be predicted. Thus we will assume that an order of magnitude estimate for the mean square change in inclination can be made by treating the fluctuations as random changes of the B_r field with a correlation time of 10^5 s .

Substituting these values and taking square roots, we find that a root mean square change in eccentricity of 0.05 will occur over 10^3 yr for $4 \mu\text{m}$ grains. This produces a spread greater than the observed width of the ring. Again, $1 \mu\text{m}$ particles would be spread an order of magnitude more.

The change in inclinations is more dramatic, spreading by 0.6° in 1 yr, or 6° in 100 yr, resulting in a ring thickness of 14,000 km. Again, smaller particles will be even more drastically affected.

We conclude that Jupiter ring particles will be strongly perturbed and scattered by electromagnetic forces on short time scale (100 yr) if they are charged to -10 V . Larger voltages are incompatible with the observed width of the ring. Given -10 V potentials, the ring must be constantly replenished, or else it is a temporary phenomenon. Alternatively, voltages may be much lower than those predicted previously.

The electromagnetic forces will be very effective in scattering particles smaller than the ring particles. Thus these forces are probably the cause of the dust cloud observed to extend above, below and beyond the jovian ring itself¹².

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Photocatalytic hydrogen production from water on Pt-free SrTiO_3 in alkali hydroxide solutions

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Semiconductor surfaces can act as photosensitizers for small molecules which on their own do not absorb solar photons. Such sensitizers can be used for the photoassisted decomposition of water to hydrogen and oxygen. Most previous work on this process has concentrated on photoelectrochemical cells using oxide semiconductor photoanodes for oxygen evolution and platinum cathodes for the evolution of hydrogen¹⁻³. We report here the sustained photogeneration of hydrogen on SrTiO_3 single crystal surfaces by a different mechanism, when no platinum coating or platinum counterelectrode is present. Hydrogen yields far exceed the monolayer amounts ($\sim 10^{15}$ molecules cm^{-2}) expected from a surface stoichiometric reaction. The reaction takes place on illumination of the crystal with band gap radiation ($h\nu \geq 3.2 \text{ eV}$) in aqueous alkaline solution. The rate of hydrogen evolution increased with increasing hydroxide concentration in the solution. We also report the use of water vapour as a reactant through saturation of a layer of a basic deliquescent compound, such as NaOH, which coated the crystal. The photocatalytic generation of hydrogen on the illuminated surface of metal-free SrTiO_3 crystals shows that strongly reductive as well as oxidative reactions can be carried out and sustained on illuminated oxide semiconductors. A wide range of photocatalytic reactions may possibly be carried out in conditions not amenable to the operation of photoelectrochemical cells by using this new mechanism.

Single crystal wafers of SrTiO_3 cut within 1° of the (111) plane were dipped in a saturated NaOH solution, dried to leave $\sim 30 \mu\text{m}$ of NaOH on the surfaces, and inserted into a stainless steel vacuum cell equipped with a sapphire window. The crystal was held in a quartz glass basket; no metal was in

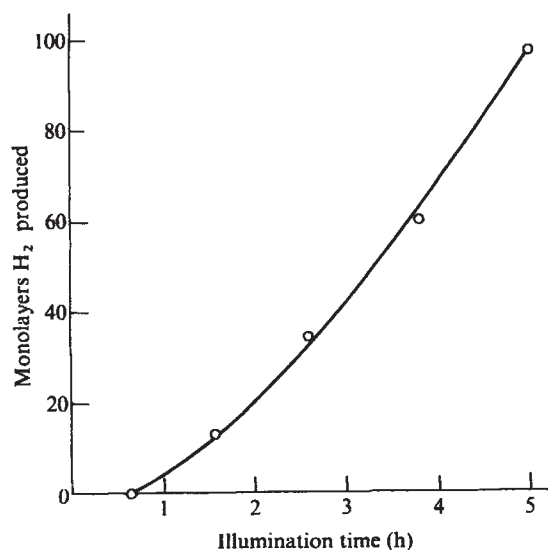


Fig. 1 Hydrogen photogeneration on a metal-free stoichiometric SrTiO_3 crystal on illumination in 20 M aqueous NaOH at 44°C .

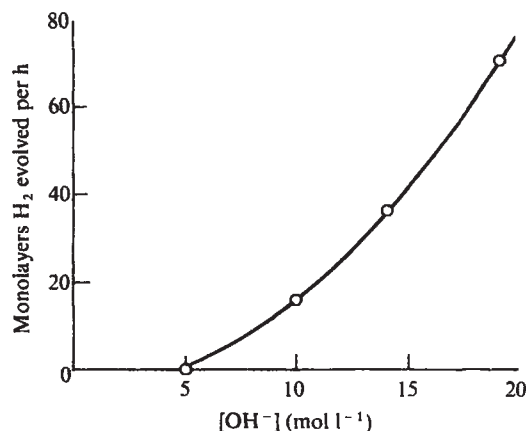


Fig. 2 Hydrogen photoproduction on a metal-free stoichiometric $\text{SrTiO}_3(111)$ crystal as a function of aqueous NaOH concentration at 44°C .

contact with it or the NaOH layer. The cell was evacuated and then backfilled with a saturation pressure of water vapour (~ 20 torr). A stainless steel bellows pump circulated the vapour and product gases around a closed loop which included a gas sampling valve. A gas chromatograph equipped with a thermal conductivity detector and a molecular sieve 5A column and operated with an argon carrier gas allowed the hydrogen concentration in the loop to be periodically measured. The crystal was illuminated with the focused, water-filtered output of a 500 W high pressure mercury lamp which provided a flux of bandgap ($h\nu \geq 3.2 \text{ eV}$) photons of $\sim 10^{16} \text{ cm}^{-2} \text{ s}^{-1}$. The use of coloured glass filters enabled the band gap and sub-band gap ($h\nu < 3.2 \text{ eV}$) radiation to be separated.

Metal-free crystals coated with NaOH and illuminated in water vapour yielded hydrogen at rates of 20–100 monolayers (1 monolayer = 1×10^{15} molecules per cm^2 illuminated surface) per hour. This range of rates was obtained on both stoichiometric (insulating) and prerduced (n-type) crystals. No hydrogen production was obtained when any of the following were absent: band gap light, saturation pressure of water vapour, SrTiO_3 , or a coating of a basic deliquescent compound such as NaOH, Cs_2CO_3 , or KOH greater than $2 \mu\text{m}$ thick. Similar results were obtained when metal-free crystals were immersed in 10 ml of thoroughly outgassed aqueous solution in a Pyrex vacuum cell and then illuminated (Fig. 1). Metal-free crystals yielded up to 50 monolayers h^{-1} of hydrogen when illuminated in 20 M NaOH at 41°C . This rate could be maintained for tens of hours. Hydrogen production was observed only in basic solutions, and the rate of hydrogen photogeneration increased with the concentration of NaOH solutions, as shown in Fig. 2. Sealing off the non-illuminated surfaces of a metal-free SrTiO_3 crystal with ultrahigh vacuum compatible epoxy caused no decrease in the rate of hydrogen photogeneration, indicating that hydrogen was produced on the illuminated surface. The epoxy was shown not to be a source of hydrogen.

For comparison, experiments were also performed with platinumized crystals. The backs of such crystals were coated with platinum through the thermal reduction of chloroplatinic acid. Platinumized, prerduced crystals yielded hydrogen at rates up to 1,600 monolayers h^{-1} when coated with NaOH and illuminated in water vapour. Rates up to 4,500 monolayers h^{-1} were observed in 20 M NaOH electrolyte. Hydrogen photogeneration on platinumized crystals showed a hydroxide concentration dependence similar to that observed on metal-free crystals. Covering the platinumized surfaces with epoxy decreased the hydrogen evolution rates to those observed on metal-free crystals. A standard SrTiO_3/Pt photoelectrochemical cell² with discrete electrodes was also

operated in the vacuum cell and gave hydrogen generation rates about five times those observed on platinized crystals, probably due to the provision of a true ohmic contact between the SrTiO_3 and platinum by use of a Ga-In eutectic.

In photoelectrochemical cells or platinized crystals of n-type semiconductors, photogenerated electrons and holes are separated by the electric field within the semiconductor depletion layer. Holes rise to the semiconductor surface where they can oxidize solution species. Electrons are driven into the semiconductor bulk and then to the platinum surfaces where reductive chemistry occurs. Hydrogen production on metal-free crystals occurs at the illuminated semiconductor surface, presumably through the generation of local reduced and oxidized centres on the crystal surface. Photoemission studies of SrTiO_3 (111) surfaces in our laboratory⁴ have shown that a reduced Ti^{3+} surface species can be regenerated by shining band gap light upon the oxygen-covered surface. The regeneration of Ti^{3+} is accompanied by oxygen photodesorption. Such reduced centres may be involved in the photocatalytic hydrogen production reported here.

High hydroxide concentrations were required for hydrogen production from metal-free crystals and also greatly increased the rates obtained on platinized crystals. Hydroxide ions at or near the surface may serve as facile hole acceptors, decreasing the probability of electron hole recombination and increasing the mean lifetime of electrons at the surface. Hydroxyl groups can be monitored on SrTiO_3 (111) surfaces by photoelectron spectroscopy⁴ and their effects on the photochemistry are being further investigated.

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Structural characteristics of fulvic acids from Continental Shelf sediments

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Fulvic acids are those components of soil organic matter that remain soluble after a dilute alkaline extract of the soil is acidified to pH 2 (refs 1, 2). This extraction procedure has been applied to marine sediments, and the organic compounds so recovered have been called marine sedimentary fulvic acids. These fulvic acids are thought to form more complex humic substances in marine sediments by condensation reactions³. However, the chemical structural compositions of marine fulvic acids have not been defined sufficiently to allow this precursor relationship to be made. Here NMR spectroscopy is used to identify more clearly the chemical structural components of some marine sedimentary fulvic acids, thus enabling a more useful examination of their relationship to more complex humic substances.

Fulvic acids of soils are thought to be composed primarily of benzenecarboxylic acid structures condensed by hydrogen bonds^{2,4,5}. Substances such as carbohydrates, proteins and lipids have been reported as components of fulvic acid

preparations^{6–8}. The presence of carbohydrates in fulvic acid preparations is not surprising considering that polysaccharides, free sugars and uronic acids are extracted from sediments by similar methods to those used for fulvic acids (by weak alkali and acid)^{7,9}.

Although the composition and structural components of marine fulvic acids have not been as extensively studied as those of soil fulvic acids, several major differences and similarities between them have been noted^{10–12}. Although the nature and amounts of various oxygen functional groups in these two types of fulvic acids are believed to be similar, there is a large difference in degree of aromaticity¹². Like soils, a significant polysaccharide content is expected for marine fulvic acids, but no definitive studies have been made to establish this fact, although Ishiwatari¹³ pointed out that fulvic acids of marine sediments are rich in total carbohydrates as determined by colorimetry.

IR spectroscopy provided some early evidence for the presence of certain functional groups of fulvic acids^{14,15} but the lack of resolution and uncertainties in peak assignments have limited its usefulness. Chemical degradations by hydrolysis, saponification, oxidation, reduction and methylation have provided valuable clues as to the structural composition of fulvic acids². The relationships between degradation products and the unaltered structures of fulvic acids, however, have always been questioned.

As non-destructive NMR techniques have recently shown promise in providing structural information on humic substances^{12,16–20} we have used this technique, as well as IR spectrometry, to examine the structural components of fulvic

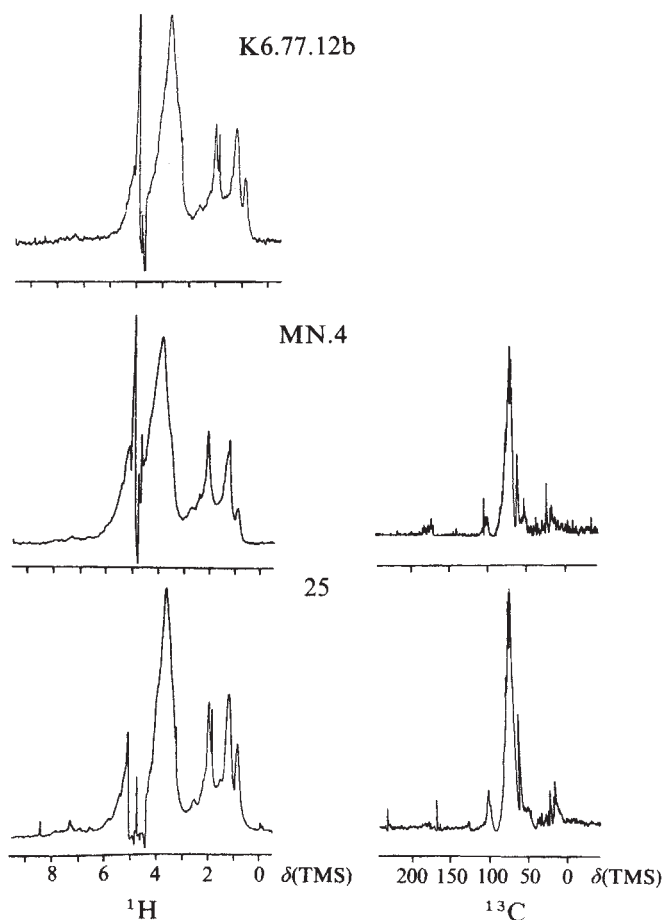


Fig. 1 The ^1H and ^{13}C spectra of fulvic acids from Continental Shelf sediments of the New York Bight. In the ^1H spectra the OH protons were decoupled. The ^{13}C spectra are spectra of approximately 10,000 transients. Chemical shifts are in p.p.m. downfield of tetramethylsilane (TMS).

acids isolated from a few samples of marine sediments. Carbohydrate analyses were also performed in an attempt to establish the polysaccharide contents of these fulvic acids.

Bottom sediments were collected from locations off the Continental Shelf of the New York Bight by a Shipek grab sampler. Samples 25 (a medium-grained sand) and MN-4 (a silty sediment) were collected ~25 nautical miles from the New York Harbor. Two other samples were collected further offshore in the New York Bight; sample K6-77-12b is a sand that was located near the shelf break, and HuG'R' is an olive-grey mud from a 270-m water depth in the Hudson Canyon.

Fulvic acids were extracted from sediments by the usual procedure² (0.5M NaOH under N₂ overnight at room temperature). They were separated from humic acids by acidification on Dowex 50W X 8 (H⁺)²⁰. The raw isolates were dialysed by ultrafiltration through an Amicon PM 10 membrane. Retentates were then lyophilized and redissolved in 4 ml of 0.5M NaOD in D₂O. Pulsed Fourier transform ¹H and ¹³C spectra were obtained with a Varian XL 100 FT NMR spectrometer operating at 100 and 25.2 MHz, respectively. ¹H spectra were obtained on a 90° pulse with 2-s acquisition times and no delay. The number of transients required for an adequate signal-to-noise ratio were generally more than 1,000. ¹³C spectra were recorded following a 90° pulse and 0.4-s acquisition time with no delay. More than 10,000 transients were usually required on ~100 mg of sample for a good signal-to-noise ratio. Because of doubts about the quantitative nature of ¹³C signals in solution spectra²⁰, we also examined a marine fulvic acid using solid-state ¹³C NMR with the cross polarization and magic-angle sample spinning (CPMASS). In these conditions, VanderHart and Retcofsky²¹ have indicated that ¹³C NMR signals are quantitative.

IR spectra were recorded on samples (~1 mg) pelletized with KBr (~300 mg) by a Perkin-Elmer model 621 spectrophotometer. Total carbohydrate content of fulvic acids were analysed by the phenol-sulphuric acid method²². Total uronic acids were determined by the carbazole method²³.

For component sugar analyses, a fulvic acid was hydrolysed in 2M H₂SO₄ at 100°C for 2 h to release free sugars which were analysed as trimethylsilyl ethers by gas chromatography²⁴.

The ¹H and ¹³C spectra for samples 25 and MN-4 are shown in Fig. 1. The organic matter in these two samples is believed to be relatively free of significant terrestrial input because the humic acids are essentially marine in origin²⁰, and hence their fulvic acids should be of a marine continental shelf or marine origin. Because of insufficient sample size, only a ¹H spectrum was obtained for fulvic acids of sample K6-77-12b. The CPMASS ¹³C spectrum of fulvic acids from sample HuG'R' is shown in Fig. 2.

The complexity and broadness of the NMR peaks show that fulvic acids are complex macromolecular compounds. Several intense signals are observed in both ¹H and ¹³C spectra: the most notable being the cluster of peaks between 3 and 4.2 p.p.m. in the ¹H spectra and 60–110 p.p.m. in the ¹³C spectra. These peaks are, respectively, characteristic of protons β to, and carbons α to an oxygen atom and are identical to those peaks obtained from carbohydrates or polysaccharides²⁵. In the ¹³C spectra, the peak at 65 p.p.m. represents the resonance of the no. 6 carbon of hexoses. Ring carbon resonances centre at 72 p.p.m. where a strong band is observed. Anomeric carbons of polysaccharides yield a signal at ~105 p.p.m. where another major peak is discernible in the fulvic acid spectra.

In the solid-state ¹³C spectrum (Fig. 2) a similar set of peaks to those in the solution ¹³C spectra is observed indicating the domination of polysaccharides. By integration, polysaccharide carbon accounts for ~52% of the total carbon. As carbon signals obtained by this cross-polarization with magic-angle spinning technique are quantitative, this confirms that polysaccharides compose more than 50% of the organic matter of marine sedimentary fulvic acids.

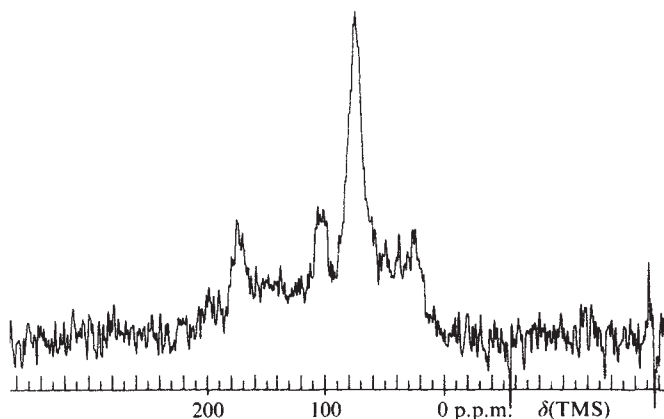


Fig. 2 The ¹³C NMR spectrum of fulvic acids from marine sediment sample HuG'R'. The spectrum represents 71,000 scans accumulated by the CPMASS technique (contact time, 2 ms; sweep width, 10 kHz; 62 kHz proton decoupling; 1,024 data points on FID; 50 s dwell time; 4 s repetition).

Although other peaks are observed in the ¹H and ¹³C spectra of fulvic acids, their contributions to the total signal in other p.p.m. regions are small. Unsubstituted aliphatic carbons and associated protons have resonance bands in the 0–50 p.p.m. and 0.6–1.5 p.p.m. regions, respectively. Methyl protons (0.9 p.p.m.) and carbons (15 p.p.m.) make significant contributions to the unsubstituted aliphatic bands. Methene protons and carbons account for peaks at 1.2 p.p.m. and 18–40 p.p.m. respectively. The relatively high proportion of methyl protons suggests that the unsubstituted aliphatic structures are of a highly branched nature.

Protons α to a carboxyl or amide functional group have chemical shifts in the 1.8–2.2 p.p.m. region. The peak at 2.0 p.p.m. indicates the presence of these protons. The presence of carboxyl or amide carbons is confirmed by the peak at ~175 p.p.m. in the ¹³C spectra (Figs 1 and 2). Quantitatively, these carbons account for ~15% of all carbons as measured in the CPMASS ¹³C spectrum (Fig. 2). Carboxyl groups in marine fulvic acids could be partly derived from uronic acid moieties associated with polysaccharides (polyuronic acids).

Although the NMR data are conclusive, IR spectroscopic and colorimetric tests have been used to confirm that polysaccharides containing uronic acids are dominant components of marine sedimentary fulvic acids. IR spectra of fulvic acids are shown in Fig. 3: these are not significantly different from published spectra of marine and terrestrial sedimentary fulvic acids¹⁵. An important band that may have been overlooked previously is the 1,050 cm⁻¹ band of polysaccharides, which is a strong absorber in these and other spectra of fulvic acids. Even soil fulvic acids (Fig. 3c) have a sizeable band in this region suggesting the presence of polysaccharides. This 1,050 cm⁻¹ band has, however, been variously interpreted: Whitby and Schnitzer¹¹ attributed Si–O stretching vibrations to this band, whereas Stevenson and Goh¹⁵ identified this band as the C–O stretch of polysaccharides. This is not surprising because ash contents of fulvic acids are not easily lowered, and silica can be tenaciously associated with fulvic acids²⁶. In fact, this may be a major reason why the high polysaccharide content of marine sedimentary fulvic acids has been overlooked.

A strong band is observed for carbonyl vibrations at 1,720 cm⁻¹ in all IR spectra, which can be attributed to carboxyl groups in fulvic acids. This band may also be attributed to the carboxyl group of uronic acids. Hence, the IR data tend to confirm the presence of polyuronic acids.

Colorimetric tests were used to calculate the total carbohydrate and total uronic acid contents of these fulvic acids. The results are presented in Table 1. In all samples, carbohydrates compose a large fraction of the fulvic acids which could be expected on the basis of the NMR data, but

Table 1 Total carbohydrate analysis of fulvic acids

Sample	% Total carbohydrate*	% Total uronic acids†
HuG'R'	38	NA
Mn-4	47	17
25	57	20

*Calculated as glucose weight relative to moisture and ash-free weights of fulvic acids.

†Calculated as glucuronic acid weight relative to moisture and ash-free weights of fulvic acids.

NA, Not analysed.

we must consider that the colorimetric technique may be biased by incomplete hydrolysis and the presence of interfering substances. The total uronic acid contents of fulvic acids (Table 1), as determined by the colorimetric carbazole method, also indicate that uronic acid moieties compose a large fraction of the fulvic acids examined. This confirms that polyuronic acids are important components of marine fulvic acids.

Component sugar determinations were made for sample 25: glucose, galactose, arabinose, mannose, xylose and rhamnose appear as the major sugars resulting from hydrolysis of this fulvic acid. Although glucose and galactose are ubiquitous components of plant and sedimentary polysaccharides^{27,28}, rhamnose, mannose, ribose and arabinose, seem to be components of microbial polysaccharides^{6,9}.

Of the studies on the nature of marine sedimentary fulvic acids, few have unambiguously identified the major structural components. Ishiwatari¹³ observed that polysaccharides composed a large fraction of sedimentary fulvic acids. Steelink²⁹, and Rashid and King¹⁰, suggested that polysaccharides or polyuronic acids could be isolated from sediments as fulvic acids or humic acids. Ernst³⁰ isolated uronic acids from fulvic acids in sediments of the North Sea. The present evidence indicates that polysaccharides containing uronic acid moieties are, by far, the most abundant components of the marine sedimentary fulvic acids examined. The solubility of polysaccharides and polyuronides in weak acidic and basic solutions has long been known³¹ and hence, in most sedimentary environments containing plant and microbial residues, these polysaccharides will be extracted. In soils where large quantities of dark aromatic humic substances are also extracted as fulvic acids, polysaccharides will probably be present in small amounts. In marine sediments, where dark aromatic substances may not be present in large amounts, polysaccharides can be the dominant components of fulvic acids, as is observed in this study. Therefore, we suggest that

polysaccharides are always extracted from sediments in low yields and that the low concentrations of dark aromatic substances determines their predominance in the fulvic acid extract.

Marine sedimentary fulvic acids are thought to be precursors of marine humic acids and kerogen^{3,32,33}. Hatcher *et al.*²⁰ have shown by ¹H and ¹³C NMR that marine sedimentary humic acids from these same samples are composed predominantly of highly branched unsubstituted aliphatic structures. Polysaccharides in marine fulvic acids would have to undergo substantial transformations to produce structures such as those found in humic acids. Although these transformations may occur, it is more likely that if fulvic acids do condense to form humic acids, polysaccharides are probably eliminated by hydrolysis and decomposition, and the minor components of fulvic acids (those observed as minor peaks in the NMR spectra) enter into this condensation process. The aliphatic and aromatic components may play an increasingly important part in this process as polysaccharides disappear.

The decreasing ratio of fulvic acids to humic acids in sediment cores from both Continental Shelf and deep-sea sediments has been attributed to the condensation of humic acids from fulvic acids³², and the ratio has been used to estimate the diagenetic maturity of humic substances in sediments. The same data sets can be explained if the fulvic acids had contained significant quantities of polysaccharides in their early depositional states and if these polysaccharides were eventually destroyed or hydrolysed. Therefore, no condensation mechanisms are needed to explain this change in ratio. Obviously, the diagenetic pathway proposed by Welte³ needs further study, and we suggest NMR as a means by which the diagenetic changes of fulvic acids and humic acids might be examined in sediment cores.

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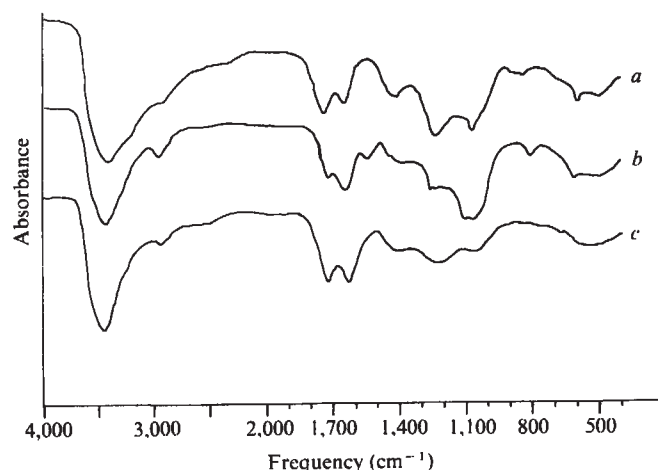


Fig. 3 IR spectra of fulvic acids from marine sediments sample K6-77-12b(a) and sample HuG'R'(b) and from a podzol soil in Prince Edward Island (c).

Metamorphism in the Troodos ophiolite: implications for marine magnetic anomalies

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The magnetic mineralogy and Koenigsberger ratio (the ratio of the remanent to induced magnetization) of basaltic rocks from the Troodos ophiolite undergo critical transformations at the zeolite–greenschist facies boundary. At this boundary the magnetic mineralogy changes abruptly from titanomaghaemite to magnetite and the Koenigsberger ratio decreases to less than 1. If similar metamorphism is occurring near the active oceanic ridges today, then the application of the two-layer model to marine magnetic anomalies may be primarily due to the degree of metamorphism and not to the mode of emplacement, intrusive versus extrusive. We show here that apparently in the Troodos ophiolite and, therefore, probably in oceanic rocks formed at spreading centres, the presence of magnetite at depth is a function of the pressure and temperature regime seen at the spreading centre.

Despite extensive efforts, deep-sea drilling has not yet reached beyond 580 m into the oceanic crust (basement). Thus scientists have had to study ophiolites, thought to be fragments of subaerially exposed oceanic crust^{1–4}, to obtain data for working models of the basic physical properties, origin and subsequent alteration of the oceanic crust. Research at the University of Minnesota palaeomagnetism laboratory has concentrated on defining the magnetic properties of ophiolites to establish boundary conditions for deeper sources of sea-floor magnetic anomalies. However, recent data also indicate that magnetic parameters can be sensitive indicators of chemical and thermal alteration^{5,6}.

The Troodos ophiolite was generally considered a fragment of Cretaceous (85 Myr) oceanic lithosphere formed at a spreading centre^{2,3,7,8}, until Miyashiro⁹ suggested that the major element chemistry was more compatible with its origin as a young island arc. The ensuing discussion emphasized that no single piece of evidence can unequivocally establish the origin of an ophiolite. Subsequent evaluation of the geological evidence combined with numerous isotopic, rare earth, major and minor element analyses suggests that the Troodos ophiolite formed within a small marginal ocean basin^{10,11}.

The basaltic rocks in Troodos have been divided into the Upper Pillow Lavas, thought to be an off-axis sequence of alkalic extrusives, and the Axis Sequence of saturated pillow lavas and sheeted dykes¹⁰. The ophiolite has undergone metamorphism from zeolite to amphibolite facies with the deeper lithologies showing the higher grade metamorphism. The transition between zeolite and greenschist facies fluctuates near the depth where the pillow lavas between dykes become scarce. This facies transition is entirely independent of the intrusive extrusive ratio⁸. Strontium and oxygen isotope analyses indicate that this alteration involved large amounts of oceanic water and was probably produced at an oceanic ridge^{12,13}.

Vine and Matthews³ measured susceptibility and natural remanent magnetization of the Troodos ophiolite lithologies; their magnetic data were categorized according to lithology.

The dykes were found to have a much lower ratio of induced to remanent magnetization (Koenigsberger ratio) than the pillow lavas and, therefore, were considered poorer contributors to magnetic anomalies. However, in the pillow lavas from the Macquarie Island ophiolite, the Koenigsberger ratio was seen to vary closely with metamorphic facies¹⁴. There the unmetamorphosed pillows exhibited high Koenigsberger ratios which contrasted with the low ratios of the greenschist facies pillows. More importantly, magnetite was discovered in the greenschist facies, while the unmetamorphosed pillows contained titanomaghaemite similar to that in oceanic pillows. These results generated speculation that magnetite may be found at depth in the basaltic rocks of the oceanic lithosphere. Our study of the Troodos ophiolite further defines the magnetic changes involved in differing basaltic lithologies and metamorphic facies, and also indicates that the greatest magnetic contrast is not between basaltic lithologies as originally suggested by Vine and Matthews³ but that it occurs between zeolite and greenschist metamorphic facies rocks.

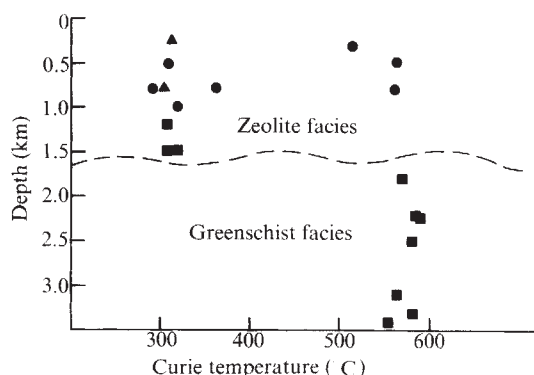


Fig. 1 The relationship between the Curie temperature of basalt samples and depth below the top of the Troodos ophiolite. The Curie temperatures near 300 °C in the zeolite facies rocks represent the onset of unmixing before the actual Curie point (probably between 350 and 450 °C) was reached.

Preliminary thermomagnetic curves of Troodos ophiolite samples from the top portion of the basaltic layer were irreversible, similar to those from oceanic pillows that have undergone low temperature oxidation^{15,16}. The titanomaghaemite in these rocks had not inverted to magnetite and an associated titanium-rich mineral and, therefore, had not undergone heating greater than 200–250 °C (refs 17, 18). Thermomagnetic curves from deeper lithologies indicated the presence of magnetite. To obtain more precise information of the location and character of these magnetic changes, further samples were obtained from J. Smewing and were carefully selected to represent increasing depths into the ophiolite. Remanent intensity and susceptibility were measured, and the Koenigsberger ratio calculated. Thermomagnetic analyses were performed on representative samples. Additional magnetic properties such as the behaviour of the natural, anhysteretic and viscous remanences during alternating field demagnetization were obtained and are reported elsewhere^{15,16}. Here we describe the drastic changes in magnetic mineralogy and decrease in Koenigsberger ratio observed in the basaltic rocks at the zeolite–greenschist facies boundary.

Figure 1 shows the sharp contrast between the character of the thermomagnetic curves above and below the zeolite–greenschist facies boundary. In the zeolite facies pillow basalts,

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sills and dykes, most of the saturation magnetization versus temperature curves were irreversible, whereas below the metamorphic boundary all were reversible with Curie points between 560 and 580 °C. However, note that the points plotted (Fig. 1) as 'Curie temperatures' near 300 °C in the zeolite facies rocks actually refer to temperatures at which unmixing occurred. More realistic Curie temperature estimates are probably between 350 and 450 °C. The thermomagnetic behaviour of samples from the zeolite facies rocks suggest titanomaghaemite whereas those in the greenschist facies are typical of low titanium magnetite.

To substantiate these conclusions, precise X-ray diffraction patterns were obtained for two dyke samples on either side of the metamorphic facies boundary. The magnetic separate from dyke rock above the boundary gave a cell edge of $8.418 \pm 0.002 \text{ \AA}$ similar to highly oxidized titanomagnetite. Electron microprobe analyses also indicated an ulvöspinel content of 51% ($\chi=0.51$). High titanium phases were not found. In contrast, the sample from below the boundary had a cell edge of $8.397 \pm 0.003 \text{ \AA}$ similar to that of magnetite. Thus, the X-ray and electron microprobe analyses verify the thermomagnetic studies.

The presence or absence of characteristic metamorphic minerals in each facies places the zeolite-greenschist boundary at about 200 °C (ref. 8). This temperature is sufficient to cause the inversion of titanomaghaemite to magnetite. If metamorphism is occurring near the present oceanic ridges, then magnetite is present at or near the depth of greenschist facies metamorphism. Thus no additional heating is required during obduction to produce the magnetite in these rocks.

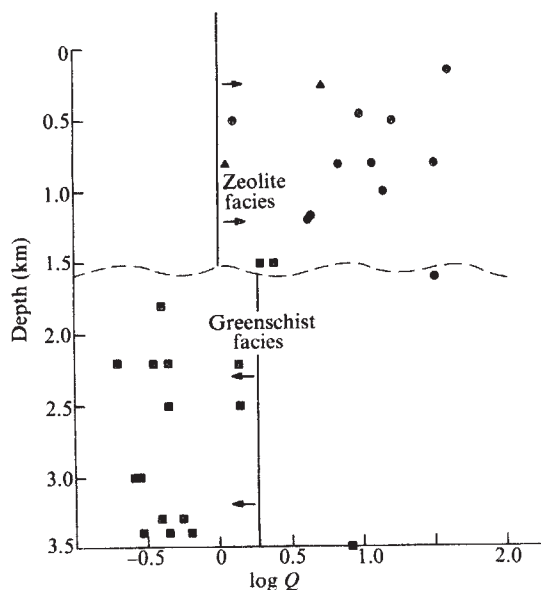


Fig. 2 Semi-log plot showing the relationship between the Koenigsberger ratio (Q) and the depth below the top of the Troodos ophiolite.

Figure 2 shows the marked change of Koenigsberger ratio at the zeolite-greenschist facies boundary. The dykes and pillows of zeolite facies all display Koenigsberger ratios greater than 1, whereas most of the greenschist facies rocks exhibit values less than 1. Among the exceptions to the latter observation is a greenschist pillow which shows an exceptionally high ratio of 31.6. The thermomagnetic curve indicates the presence of maghaemite which may be of post-obduction origin⁵. Therefore, the high Koenigsberger ratio and

thermomagnetic curve of this sample are not considered as characteristic of greenschist facies oceanic crust. The decrease of the Koenigsberger ratio generally reflects the behaviour of the remanent intensity near the zeolite-greenschist metamorphic boundary, whereas the susceptibility remains relatively constant. In addition, the stability, defined by the alternating field at which half of the remanent magnetization is destroyed, showed little variation. However, the greenschist facies basalts did acquire a large amount of viscous remanence, as did these same rocks from the Smartville and Othris ophiolites^{5,6,15}.

Figure 2 indicates that the magnetic signature of the Troodos basalts depends greatly on the extent of metamorphism. Although the Koenigsberger ratio also seems to be slightly affected by the mode of emplacement, that is, pillow versus sheeted dykes, the change of values is not as distinct as that seen at the metamorphic facies boundary. More compelling is the sudden change in magnetic mineralogy at this same facies boundary, suggesting that it may influence the Koenigsberger ratio.

The importance of the zeolite-greenschist metamorphic boundary indicated by the magnetic data from Troodos suggests that grouping magnetic data according to basaltic lithology as is currently practised³, may be inappropriate in many cases and may mask important trends. More importantly, the boundary between the zeolite and greenschist metamorphic facies should be of primary consideration when modelling marine magnetic anomalies. The actual amount which the greenschist facies basalts contribute to the magnetic anomaly pattern may not be insignificant but would depend on their thickness relative to the zeolite basalts.

The remarkable transition in magnetic properties from the zeolite to greenschist facies rocks is paralleled by a substantial increase of 1.7 km s^{-1} in seismic velocities measured at the outcrop¹⁹. This increase is attributed to an increase in rigidity caused by the presence of quartz in the greenschist facies rocks⁸. However, the direct application of these seismic results to the oceanic crust is hindered by the velocity changes caused by the subaerial exposure of the Troodos ophiolites, the accompanying release of hydrostatic pressure, and the onset of weathering. Thus, velocities measured in the laboratory under hydrostatic pressures are as much as 1.0 km s^{-1} faster, but the great difference in velocities between the metamorphic facies still seem to be present²⁰. Multiple layers in oceanic layer 2 are now detectable by sonobuoy techniques. If this seismic method should succeed in detecting metamorphic boundaries, then this data, plus the Troodos results regarding magnetic contrasts across this boundary, would offer important constraints for magnetic anomaly modelling of the ocean floor.

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Antlers, body size and breeding group size in the Cervidae

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The tendency for large deer species to have relatively big antlers for their body size has been explained as the result of an inevitable evolutionary trend leading to increased antler size¹⁻⁴, of linkage between genetic factors influencing body size and relative antler size⁵, of the increased need for larger deer to lose heat⁶ and of an increased reliance of larger deer species on displays to avoid fighting⁷. Here we test an alternative hypothesis: that large deer species tend to be more polygynous than smaller ones and that intense inter-male competition in large species has led to the development of relatively large antlers. Analysis of comparative data⁸⁻⁴² confirms two predictions based on this hypothesis: (1) that large deer species form bigger breeding groups than small ones; and (2) that deer which form large breeding groups have relatively larger antlers for their body size than those that form smaller ones. However, antler size is not proportional to body size among species that form breeding groups of similar size, indicating that other factors must also be involved.

The distribution of antler size across deer species poses a problem to evolutionary explanation. Small deer species, such as muntjac or brocket, have small antlers relative to their body size while larger species, like red deer or reindeer, have relatively large ones⁵⁻⁷. In the early part of this century, the large antlers of the deer was usually explained as the result of a directional evolutionary trend¹⁻⁴. Huxley⁵ contested this view, arguing that the trend occurred because the genetic factors responsible for large body size were linked to those responsible for relatively large antler size and that selection for increased body size automatically increased the relative size of antlers. More recently, two functional explanations have been suggested. First, Gould⁷ has argued that large deer species seldom fight—settling disputes instead by antler displays—and that this has caused selection to favour large antlers. However, the available information indicates that fights between competing males are not uncommon^{43,44} and there is no firm evidence that individuals assess their opponents by the size of their antlers or that individual differences in antler size are closely related to fighting ability or dominance when differences in age and body size are taken into account: none of the studies relating antler size to dominance or fighting ability⁴⁵⁻⁴⁷ have controlled adequately for the effects of age and body size, which are commonly correlated with social rank^{43,48}. Moreover, as stags rarely fight outside the rut, and during the rut their fighting ability declines as their body condition deteriorates⁴³, it would be an inefficient assessor that judged its opponent principally on traits that were insensitive to short-term changes in condition⁴⁹.

Second, Stonehouse⁶ has argued that large deer may have relatively large antlers for thermoregulatory reasons: during their period of growth, antlers may assist heat loss and larger species may have developed relatively large antlers on account of their reduced surface to volume ratios. But this explanation is unsatisfactory because of the obvious integration of antler growth with the sexual cycle^{50,51}, the lack of a close

Table 1 Measures of mean shoulder height and mean record antler length used in the analysis

Cervidae		Shoulder height (cm)	Antler length (cm)	Breeding group size	Refs
Muntiacinae					
<i>Muntiacus muntjak</i>	Indian muntjac	56.7	17.6	A	10
<i>M. reevesi</i>	Reeve's muntjac	44.5	8.9	A	11
Cervinae					
<i>Dama dama</i>	Fallow	91.0	72.9	C	12
<i>Axis axis</i>	Chital, Spotted	93.5	93.0	C	13
<i>A. porcinus</i>	Hog	68.6	49.2	C	13, 14
<i>A. kuhlii</i>	Bawean, Kuhl's	68.6	24.8	A	15
<i>Cervus duvauceli</i>	Swamp	120.0	95.2	C	13, 16
<i>C. elaphus</i>	Red	121.9	93.6	C	17, 18
<i>C. canadensis</i>	Wapiti	160.0	147.4	C	19, 20, 21, 40
<i>C. eldi</i>	Eld's, Thamin	124.4	94.3	C	22, 23
<i>C. nippon</i>	Sika	80.9	56.7	C	24, 25
<i>C. unicolor</i>	Sambar	150.7	109.8	B	14
Elaphurus					
<i>elaphurus davidianus</i>	Père David's	116.8	73.7	C	26
Odocoileinae					
Odocoileus					
hemionus					
	Mule, Black-tailed	101.2	71.0	B	27, 28
<i>O. virginianus</i>	White-tailed	98.2	65.6	B	29, 41
Capreolus					
<i>capreolus</i>	Roe	69.4	28.3	B	30, 31, 42
<i>Alces alces</i>	Moose	205.7	108.0	B	32, 33, 34
Rangifer					
<i>tarandus</i>	Reindeer, Caribou	120.0	117.2	C	35, 36
Hippocamelus					
<i>bisculcus</i>	Huemul, Guemal	96.9	24.8	B	37
<i>H. antisenensis</i>	Huemul, Guemal	86.4	26.4	C	38
<i>Mazama americana</i>	Red brocket	69.8	11.5	A	9
<i>M. gouazoubira</i>	Brown brocket	56.0	11.5	A	9
M. rufina					
	Little red brocket	43.6	9.4	A	9
Pudu pudu					
	Pudu	35.5	7.5	A	39

Species were classified into three groups on breeding group size: A ≤ 2 ; B, 3–5; C ≥ 6 animals. Refs 8 and 9 were also used for each species.

association between seasonal variation in temperature and the period for which the antler is in velvet, and because of the tendency for species with sub-arctic ranges to have the largest antlers^{44,46,52}.

This paper tests a third functional explanation of the relationship between body size and relative antler size: that inter-male competition is more intense among large deer species than among small ones because large deer tend to form bigger breeding groups⁵³, and that sexual selection has consequently favoured the greater development of fighting apparatus in larger species. This argument rests on three assumptions: that antlers are regularly used in fighting; that gross increases in antler size improve their effectiveness in defence or attack; and that the size of breeding groups is related to the intensity of inter-male competition. There is now much evidence that fights between breeding males are common in polygynous deer species and that antlers are important in these fights, enabling animals to grip their opponents during pushing contests and protecting their heads and shoulders^{43,44}. Studies of fighting tactics indicate that the large, complex antlers of the bigger deer species probably form a safer guard against injury than the smaller and simpler ones typical of smaller species^{54,55}. Evidence from other groups of vertebrates suggests that inter-male competition increases with the size of breeding groups^{56,57} and that male weaponry and other characteristics which improve combative ability (including increased body size) are more developed where inter-male competition is strong^{57,58}.

If this explanation accounts for the interspecific allometry in antler size among deer, two predictions should be fulfilled: (1) larger deer species should form bigger breeding groups than smaller ones and relative antler size should consequently be greatest among species forming large breeding groups; and

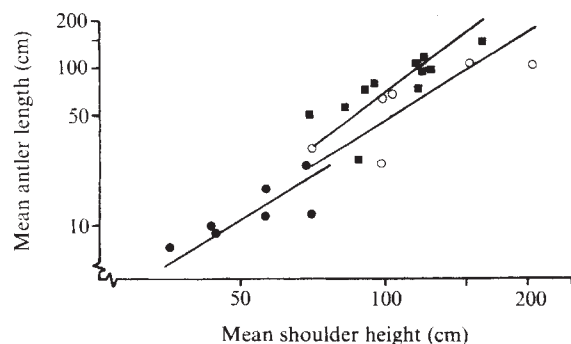


Fig. 1 Mean (record) antler length plotted on mean shoulder height for different deer species (see Table 1). ●, group A; ○, group B; ■, group C. Lines show major axes (see text).

(2) among species forming breeding groups of similar size, antler size should be directly proportional to body size.

To test these predictions, data on body size, antler size and breeding group size, were taken from the literature (Table 1). Our measure of body size was mean shoulder height and, of antler size, the mean length from coronet to top point of all heads, for which measurements are provided by Ward⁸. The latter measure had two important disadvantages: although gross differences in antler length among cervids reflect variation in antler volume and complexity, some genera (for example *Alces*) have antlers that are relatively short for their volume; and there is a danger that the use of estimates of average shoulder height and record antler lengths will increase the slope of antler size on body size (though previous analyses indicate that this effect is probably unimportant⁷). Species were allocated to three categories according to the reported size of groups during the breeding season: A, group size ≤ 2 ; B, group size 3–5; C, group size ≥ 6 .

Group sizes used to categorize species included both males and females. However, sex ratios in breeding groups are typically female-biased and, in the sample of species for which estimates of the average number of females in breeding groups were available, this measure correlated well with total group size ($r_s = 0.991$, $n = 7$, $P < 0.001$). The three categories we have used disguise many differences in behaviour between species, but the available information on breeding systems of cervids does not permit finer classification. To investigate relationships between antler length and shoulder height in different groups, we used major axis analysis⁵⁹ on the logarithmically transformed data. To compare relationships between groups, we used the maximum likelihood method for testing heterogeneity of slopes⁶⁰.

Within all three groups, as well as across the sample as a whole, there is a close relationship between antler length and shoulder height (see Table 2). The analysis confirms that, as our hypothesis predicts, breeding group size is positively correlated with shoulder height ($r = 0.433$, $t = 2.15$, d.f. = 22, $P < 0.05$). As no heterogeneity of slopes was revealed by the maximum likelihood analysis ($\chi^2_2 = 0.201$) we have imposed the common slope (2.082) through the mean for both variables and sought differences in weighting on the minor axis (a process analogous to testing for differences in elevation using

regression analysis). Significant differences exist between groups A and C ($t = 2.140$, d.f. = 16, $P < 0.05$) and B and C ($t = 2.200$, d.f. = 15, $P < 0.05$), but not between groups A and B ($t = 0.304$, d.f. = 11, not significant). As predicted, antler size is larger in group C than in groups A or B and within-group slopes tend to be shallower than the overall slope (see Table 2).

Contrary to prediction, the allometric relationship between shoulder height and antler length remains within all three groups (Table 2). This may be because of the coarseness of our categorization of breeding systems, but it seems more likely that other factors are involved. However, before we need have recourse to non-adaptive explanations, there are three functional hypotheses which should be considered: (1) Larger deer can deal heavier blows than smaller ones, their attacks are likely to be more damaging and males may consequently be selected to develop a more effective guard⁵⁵. (2) If larger deer species have longer lifespans than small ones⁶¹, males may engage in a greater number of fights during their lifetimes and may have a higher overall risk of being injured. For this reason, they may invest in larger and more effective antlers. (3) Larger species tend to occur in northern countries where the energetic costs of antler production may be reduced by seasonal superabundance of food supplies^{62, 63}.

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Table 2 Analysis of relationships between $\log_e$ (antler length) and $\log_e$ (shoulder height) in cervids (see Table 1).

Group	A	B	C	Overall
Sample size	7	6	11	24
Correlation between $\log_e$ (antler length) and $\log_e$ (shoulder height)	0.776	0.805	0.769	0.903
b (slope)	1.907	1.888	2.265	2.356

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Subdivision of nature reserves and the maintenance of species diversity

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The roles of area—its size, shape and possible subdivision—in the conservation of species is an important but controversial topic^{1–5}. The fundamental assumption is that nature reserves act as habitat islands in an inhospitable sea of environment that has been modified by man, and thus that the empirical and theoretical findings of island biogeography are pertinent. Empirical studies of oceanic islands and continental habitat islands show that larger areas hold more species. The relationship between these two variables is such that a 100% increase in area produces roughly a 25% increase in species. Thus, two small habitat islands which have more than this 25% difference in their species composition, hold more species than a single habitat island of the same total area. This is probably due to microhabitat differences between these two smaller habitat islands. Yet, even against a constant ecological background, stochastic fluctuations ('turnover') of species due to random colonization and extinction⁶ will cause inter-island variation in species composition.

In the preceding article Higgs and Usher⁷ have shown that three, presumed homogeneous and similarly-sized quarry reserves have sufficient differences in species composition to overcome the area effect and to favour subdivision as the best strategy for maximization of diversity. We have found the same effect in 13 New Hebrides islands. This trend is consistent with equilibrium island biogeographic theory.

We choose for our empirical study 13 central New Hebridian islands with very much the same lowland rainforest habitat for the 52 bird species that occur on the archipelago. From the species lists of the 13 islands^{8–9}, we can determine the number of distinct species on each of the 78 possible island pairings and relate this to the corresponding total area of each pair. The regression line for this relationship can be compared to the species-area regression for single islands (Fig. 1): two paired islands hold just slightly (about 5–10%) more species than are expected to be contained on a single island of the same total area, in qualitative agreement with Higgs and Usher.

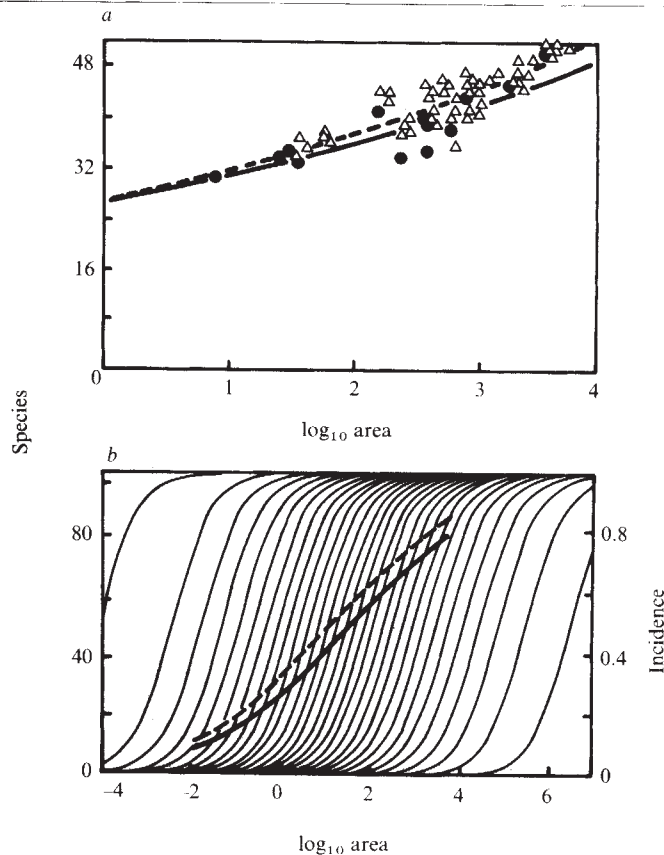


Fig. 1 *a*, New Hebrides species-area relationships. ●, individual species number versus area for 13 central islands, the regression line for which (solid line) is $S = 26.6A^{0.0666}$; △, the total number of distinct species on pairs of islands versus their total area; the regression for this (dashed line) is $S = 26.9A^{0.072}$. *b*, the light lines are an example of a set of area-dependent incidence functions (on the right-hand abscissa) whose parameter b_i is distributed log-normally with a mean and variance chosen to maximize the fit of $\sum J_i(A)$, given by the solid line, to the empirical Solomon Island S - A relationship. The dashed line is expected number of species on a pair of identical islands whose total area is A .

Our analysis of this result is based on the classical equilibrium theory⁶ and on the additional patterns revealed in Diamond's extensive study of the Pacific avifauna¹⁰. By analysing the area requirements of individual species, he found that the probability of a species' occurrence on an island, which he called its incidence, increases sigmoidally with increasing logarithm of island area. We¹¹ have since demonstrated that such incidence curves should have the form $J_i(A) = 1/(1 + a_i/A)$, where J_i is the incidence of the i th species, A is island area and a_i is a species-specific ratio of extinction rate to colonization rate. A lognormal distribution of the values of a_i for the T possible mainland pool species can be fitted by regression analysis to explain the observed distribution of the Solomon Island avifauna (Fig. 1). The number of species expected for any given area is the sum of all the incidence functions evaluated at that area: $S = \sum J_i(A)$. The number of species expected on two islands of half the area is: $S' = \sum (2J_i(A/2) - J_i(A/2)^2)$, where the $J_i(A/2)^2$ term subtracts out the species expected to occur on both islands. With this theoretical treatment, which is based on the data for the Solomon Islands, we can show that S' is ~10% greater than S over the entire range of relevant areas, which closely agrees with the New Hebrides pattern (Fig. 1).

There are thus two compensatory effects dependent on island size: lumping of areas produces more species per island, but subdivision of area produces a more complete sampling of the possible colonist species for islands of that size. Our

analysis shows that division in twos produces increased species diversity. But the even more thorough sampling of still smaller but more numerous islands quickly proves suboptimal: 10 islands of a tenth the total area have fewer species than a single whole island of the same area.

Different species will tend to be preserved by these different strategies. More species with intermediate incidence curves (relatively moderate area requirements) will be picked up with subdivision, while the species with high incidence functions (large area requirements) will only be found with lumping. Thus, truly enlightened conservation practice depends not only on knowledge of the ecology of individual species, but some particular evaluation, whether based on aesthetics or economics, of the value of each species.

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Should nature reserves be large or small?

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Conservation of wildlife is one form of land use that competes with agriculture, forestry, urban development and outdoor recreation for an extremely limited supply of land. Conservationists are often faced with having to make decisions of priority, and the question arises as to whether it is better to have the area allocated for conservation in one large unit or in several smaller units. The scale of these units will vary both with different habitats and with different species being conserved: thus, in the tropics the area being considered is likely to be of the order of thousands of hectares, whereas in the industrialized world the areas may be only a few hectares in extent. The problem of how best to use limited resources becomes critical both to amateur conservation organizations (whose 'shopping lists' for reserves are long, but whose financial resources are limited by the extent of their charitable status) and to national organizations such as, in the UK, the Nature Conservancy Council (whose ability to support management agreements under Section 15 of the 1968 Countryside Act is limited by an overall budget). The question is: should such limited resources be devoted to the conservation of a few large sites or to two or three times as many smaller sites? The following argument shows that the proportional overlap of species is the critical factor, and data indicate that a number of small reserves have more species than a single large reserve.

Theoretical studies on nature reserves^{1–3} all indicate that size is an important design consideration. These studies advocate larger preserves, although some of the disadvantages of large size have been raised^{4,5}. In studies on conservation evaluation, size is often listed with diversity or species richness⁶. Both these criteria seem to be given equal weight in the final assessment of conservation value, even though the theoretical studies indicate that such criteria may be closely correlated. Such a correlation arises from the species–area relationship, which has been used to

justify conserving larger areas. It has, however, been pointed out^{4,5}, although not formalized, that the species–area relationship may favour the setting up of several smaller reserves. The predictions that can be made from the species–area curve on the total number of species conserved on two reserves depend on the proportion of species that occur on both reserves. Such predictions consider only the total number of species and not the possibility of future trends due to extinction or immigration (often associated with successional processes).

Suppose that the limited financial resources allow for the conservation of only A units of area. The option facing the designer of a reserve system may be between one 'large' area, of A units, or two 'small' areas of pA and $(1-p)A$ units ($0 < p < 1$). Assuming a species–area relationship ($S = CA^z$) for organisms on nature reserves^{7,8}, the number of species occurring on one large block, S_1 , is given by

$$S_1 = CA^z \quad (1)$$

where C and z are positive constants. The total number of species on the two smaller pieces is given by

$$S_2 = C(pA)^z + C\{(1-p)A\}^z - V \quad (2)$$

where V is the number of species common to the two smaller areas, that is, the overlap. If the same number of species are conserved in either situation, then $S_1 = S_2$ and

$$V = \{p^z + (1-p)^z - 1\}CA^z \quad (3)$$

If V exceeds this value, more species will be conserved on one large reserve, whereas if V is smaller the two smaller reserves conserve more species (ignoring possible future extinctions). Defining the proportional overlap on the two smaller reserves as

$$P_v = V/S_2 \quad (4)$$

which is the same as the Jaccard coefficient used in classificatory studies, and substituting equations (2) and (3) in equation (4), we obtain

$$P_v = p^z + (1-p)^z - 1 \quad (5)$$

a function which is shown in Fig. 1. It will be seen that the value

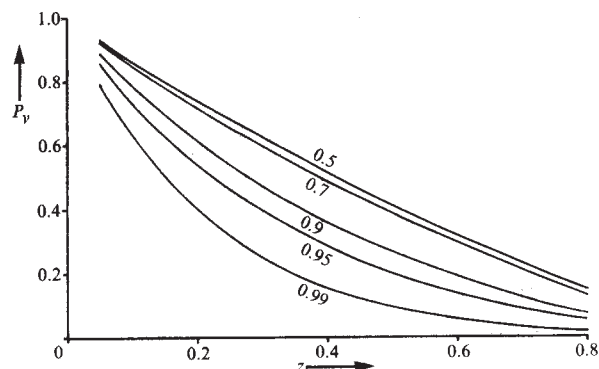


Fig. 1 Isoclines of the function relating P_v to z , equation (5). Values of p are given on each isocline.

of p has relatively little effect on the relationship between P_v and z . Thus, for z of about 0.3 (ref. 8) and with the two areas not differing by more than about half an order of magnitude in size, there will be more species on two reserves if the proportional overlap between them is less than approximately 0.6.

Studies on three quarry nature reserves (Table 1) indicate that, to maximize the number of higher plant species on nature reserves of a similar geological type, there is a benefit to having a number of smaller areas, although some of these benefits are extremely small (the estimated P_v values from field sampling are all less than the values calculated by equation (5)). There is thus no evidence, purely to house numbers of species on reserves, to suggest that one large chalk quarry is the optimal strategy.

Table 1 A comparison of three quarry nature reserves in the chalk (Cretaceous) of the Yorkshire Wolds (KCP, Kiplingcotes Chalk Pit; RBQ Rife Butts Quarry; WQ, Wharram Quarry)

Reserves compared	p	P_v estimated field lists	P_v calculated from equation (5)
RBQ, WQ	0.94	0.40	0.44
WQ, KCP	0.59	0.43	0.65
RBQ, KCP	0.92	0.45	0.48

A value of $z = 0.276$ (ref. 7) has been used. If P_v from field lists is less than the calculated P_v , then two smaller reserves are favoured.

Limestone pavements in the Yorkshire Dales National Park show a low, but statistically significant, value of $z = 0.086$ (ref. 9), although similarly low values of z have been obtained with New Hebridean island birds¹⁰. Comparisons of 16 limestone pavements, with areas between 2 and 5 hectares, gave the values of P_v shown in Fig. 2. In all 120 comparisons, a greater number

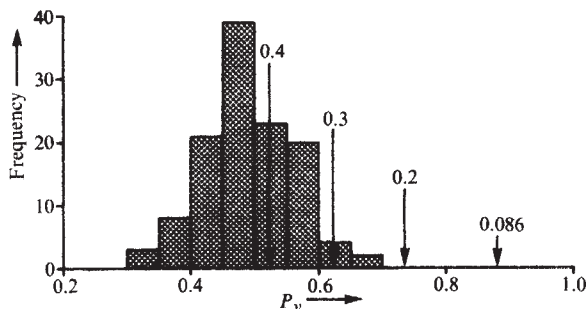


Fig. 2 A histogram of 120 P_v values in the comparison of 16 limestone pavements with areas of between 2 and 5 hectares. Two smaller pavements contain more species if P_v is less than the value indicated for an appropriate z value ($z = 0.086$ for these pavements). Values of z are indicated on arrows.

of species would be conserved if two small pavements were preserved rather than one larger pavement equal in area to that of the two smaller pavements. Even if a more typical z value between 0.2 and 0.4 was selected, there would still be a balance in favour of conserving, for the diversity of their species, several small pavements rather than a few large pavements. Qualitatively similar conclusions seem to hold for other habitats which are the focus of nature conservation management: these include soft coastal habitats in Scotland and lowland heaths in Yorkshire.

However, the number of species at one time is only one factor to be considered when designing a system of nature reserves. Higgs¹¹ discusses the limitations of applying the ideas of island biogeography to nature reserve design. Although these ideas are useful in considering the diversity of species, and possibly also in suggesting future trends in numbers of species with possible extinctions, the aims of managing the reserve, the biological attributes of the organisms being conserved and the practicalities of management, are also important considerations.

Increased pulmonary α -adrenergic and reduced β -adrenergic receptors in experimental asthma

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The role of adrenergic mechanisms in the pathogenesis of asthma is controversial. Increased airways resistance in asthmatics is reversed by β -adrenergic receptor agonists such as isoprenaline, and it has been suggested that β -adrenergic activity is diminished in this condition¹. This is supported by studies showing reduced metabolic responses to β -adrenergic agonists^{2,3} and fewer lymphocyte β -adrenergic receptors in asthmatics than in normal subjects⁴. However, the main contributory factor to diminished β -receptor responsiveness is probably a history of treatment with β -adrenergic agonists, resulting in tachyphylaxis^{5,6}. Nevertheless, similar but less pronounced changes have been observed in untreated asthmatic patients⁷. α -Adrenergic agonists produce bronchoconstriction in asthmatic patients, but not in normal subjects^{8,9}. Similarly, *in vitro* studies show α -adrenergic receptor-mediated constriction of bronchial smooth muscle from patients with increased airways resistance, but not from normal controls^{10,11}. In addition increased α -adrenergic receptor-mediated responses in vascular and pupillary smooth muscle have been reported in asthmatics¹². Using radioligand binding techniques, we have investigated the possibility that changes in numbers of α - and β -adrenergic receptors or their affinity are associated with the changes in adrenergic responsiveness observed in asthma. We report here that increased α - and fewer β -adrenergic receptors were observed in pulmonary homogenates of an animal model of chronic asthma than in those from controls.

In this model of experimental asthma, affected animals have many characteristic symptoms, including wheezing, dyspnoea and airway hypersensitivity¹³. Guinea pigs were sensitized to ovalbumin by intraperitoneal injection, and then exposed daily to an ovalbumin aerosol for 4 weeks. They developed dyspnoea, coughing and cyanosis during and after each 45-s exposure. Control animals were exposed to saline aerosol only. A lung membrane homogenate was prepared, and a radioligand binding assay was performed as before¹⁴. β -Adrenergic receptors were identified with ³H-dihydroalprenolol (³H-DHA), and specific binding (>90% of total) was defined as that displaced by 1 μ M ($\pm$)propranolol. Stereospecificity of binding of β -adrenergic receptors was confirmed with (–)propranolol ($K_i = 2.3$ nM) which was 200 times more potent than (+)propranolol as an inhibitor of ³H-DHA binding. Butoxamine, a selective β_2 -adrenergic antagonist, was more potent ($K_i = 620$ nM) than the selective β_1 -adrenergic antagonist atenolol ($K_i = 86$ μ M) as an inhibitor of ³H-DHA binding to β -adrenergic receptors in this pulmonary homogenate. α -Adrenergic receptors were identified with ³H-prazosin, and specific binding (60–70% of total) was taken as that displaced by 10 μ M phentolamine. The specificity of binding indicated that α_1 -adrenergic receptors (postjunctional) were identified with ³H-prazosin¹⁴.

There was a significant ($P < 0.001$) difference in binding to α -adrenergic receptors (Fig. 1), with maximum binding (B_{max}) of ³H-prazosin of 47.3 ± 3.7 fmol per mg protein (mean $\pm$ s.e.m.; $n = 6$) in controls and 99.5 ± 8.5 fmol per mg protein in sensitized animals ($n = 6$). There was no difference in binding affinity with equilibrium dissociation constant (K_D) values of

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0.51 ± 0.04 nM in controls, and 0.59 ± 0.05 nM in sensitized guinea pigs. There was a small, but significant ($P < 0.05$), difference in ^3H -DHA binding to β -adrenergic receptors (Fig. 2), with B_{max} values of 1,482 ± 96 fmol per mg protein in controls, compared with 1,185 ± 51 fmol per mg protein in sensitized animals. The K_D values for ^3H -DHA binding were not significantly different between the two groups (0.75 ± 0.07 nM in controls and 0.73 ± 0.08 nM in sensitized guinea pigs). Activation of adenylate cyclase mediated by β -adrenergic receptors was measured in pulmonary homogenates of control and sensitized guinea pigs (Fig. 3). There was no significant difference in maximum activation by isoprenaline (73 ± 7% in controls compared with 67 ± 4% in sensitized animals), or in the concentration of isoprenaline producing half-maximum activation in control (110 ± 8 nM) and sensitized animals (100 ± 6 nM).

Lung homogenates of sensitized animals that had been exposed chronically to antigen aerosol had twice the

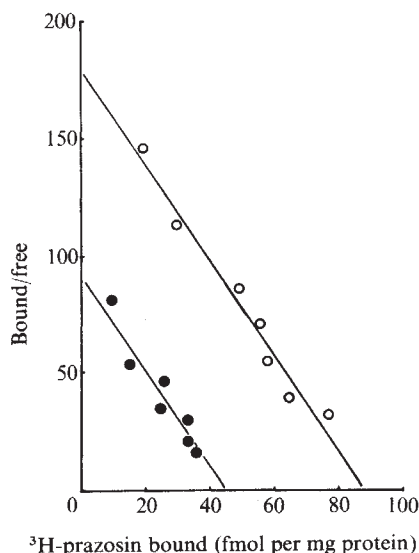


Fig. 1 Scatchard plot of specific ^3H -prazosin binding to lung membranes of typical control (●) and sensitized (○) animals. K_D = 0.48 nM and B_{max} = 44 fmol per mg protein for one of the control animals; 0.49 nM and 88 fmol per mg protein for one of the sensitized animals. The slope was determined by linear regression analysis. The abscissa intercept gives the maximum binding capacity (B_{max}), and 1/slope gives the dissociation constant (K_D). Male Dunkin-Hartley guinea pigs were sensitized by intraperitoneal injection of 1 mg ovalbumin in 1 ml saline. Two weeks later animals were exposed daily to an aerosol containing 1% ovalbumin, delivered by a Wright nebulizer into a specially designed box. Each animal was exposed for 30–60 s, 5 days each week for 4 weeks. In some cases the resultant dyspnoea necessitated revival with 100% oxygen. Control animals were injected with the saline carrier, and exposed to a saline aerosol only. The lungs were removed after cervical dislocation, homogenized in 0.32 M sucrose, and centrifuged at 500g at 4°C to remove undisrupted cells and nuclei. The supernatant was centrifuged at 50,000g at 4°C for 15 min, and the resulting pellet was washed by resuspension in 50 mM Tris-HCl buffer, pH 7.4 (assay buffer). After a second centrifugation at 50,000g, the pellet was suspended in assay buffer with a concentration of approximately 1 mg protein per ml. This membrane preparation was incubated with increasing concentrations of ^3H -prazosin (specific activity 33 Ci mmol $^{-1}$; a gift from Dr P. Greengrass, Pfizer, Sandwich) at 25°C for 15 min. Nonspecific binding was determined by incubation in the presence of 10 μM phentolamine. Incubations were terminated by filtration under reduced pressure, and the membranes were isolated on Whatman GF/B glass fibre filters. The filters were washed three times with 6 ml of the assay buffer at 0°C. Filters were counted in a Packard liquid scintillation spectrometer with an efficiency of 40%.

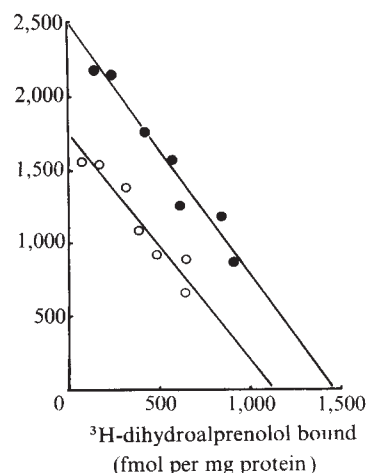


Fig. 2 Scatchard analysis of ^3H -DHA binding to lung membranes in typical control (●) and sensitized (○) guinea pigs. K_D = 0.58 nM and B_{max} = 1,453 fmol per mg protein for one of the control animals; 0.64 nM and 1,135 fmol per mg protein for one of the sensitized animals. Specific binding was defined as that displaced by 1 μM (+)propranolol. Methodology was as described in the legend to Fig. 1. The ^3H -DHA (specific activity 59 Ci mmol $^{-1}$) was supplied by the Radiochemical Centre.

concentration of α_1 -adrenergic receptors, and a marginally lower concentration of β -adrenergic receptors than controls. No significant difference was observed in β -adrenergic regulation of cyclic AMP synthesis, suggesting that in this

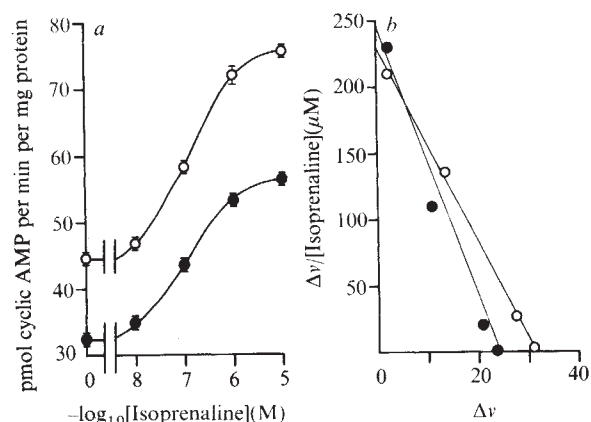


Fig. 3 *a*, Activation of adenylate cyclase (ATP pyrophosphatase; EC 4.6.1.1) by increasing concentrations of (–)isoprenaline in lung homogenates of typical control (●) and sensitized (○) guinea pigs. *b*, Eadie-Hofstee plots of these results where Δv is the increase in adenylate cyclase activity at any particular (–)isoprenaline concentration. Lung homogenates were taken from experiments described in the legend to Fig. 1, after the 500g centrifugation. Adenylate cyclase activity was determined by a modification¹⁶ of the method C of Salomon *et al.*¹⁷. Incubations of 100 μl contained 50 mM Tris-HCl buffer, pH 7.4; 5 mM MgCl $_2$; 85 mM sucrose; 20 mM creatine phosphate, disodium salt (Sigma); 10 IU creatine kinase, 150 IU per mg protein (ATP creatine *N*-phosphotransferase; EC 2.7.3.2, Sigma); 1 mM cyclic AMP, sodium salt (Sigma); 0.25 mM Ro20-1724 (phosphodiesterase inhibitor, Roche); 0.5% ethanol; 1 mM [α - ^{32}P]ATP (3 μCi , Radiochemical Centre); 0.1% ascorbate; 10 μM pargyline (Sigma); and 280–350 μg homogenate protein. Reaction mixtures were incubated at 37°C for 12 min. The concentration of ^{32}P -cyclic AMP in each incubation was determined by liquid scintillation spectrometry, with correction for recovery of ^3H -cyclic AMP after the chromatographic separation of ATP and cyclic AMP. The production of ^{32}P -cyclic AMP was proportional to protein concentration within the range 50–460 μg of homogenate protein per reaction mixture; similarly ^{32}P -cyclic AMP synthesis increased linearly for 15 min.

animal model altered sensitivity to β -adrenergic agonists is not important. However, the ratio of α : β -adrenergic receptor binding sites was 1:12 in sensitized animals and 1:30 in controls, which provides a possible explanation for altered sensitivity to adrenergic agents in asthma, and suggests a mechanism of airway obstruction in this experimental model involving responses mediated by α -adrenergic receptors.

The cellular location of the receptors identified remains obscure because we used homogenates of whole lung which included airways, blood vessels and parenchyma. Increased numbers of α -adrenergic receptors might be localized to bronchial smooth muscle, and mediate muscle contraction and increased airway resistance. Increased sensitivity to α -adrenergic agonists has been demonstrated previously *in vitro* in human bronchial smooth muscle after exposure to histamine¹¹. An alternative site for increased α -adrenergic receptors is the mast cell, where α -adrenergic agonists facilitate release of histamine¹⁵. Increased release of histamine might then result in a secondary hypersensitivity of bronchial smooth muscle to α -adrenergic agonists, as suggested above. These results are preliminary, but support the hypothesis that altered adrenergic responses in asthma may be mediated by changes in numbers of receptors.

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(-)-N-(Chloroethyl)norapomorphine inhibits striatal dopamine function via irreversible receptor binding

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β -Haloalkylamine derivatives such as phenoxybenzamine are thought to irreversibly inactivate noradrenaline receptors by a process involving the formation of a reactive ethyleniminium cation which is followed by ring scission yielding the reactive carbonium ion which can then react further with a nucleophilic centre located on the receptor. Further study of catecholamine function has awaited the development of similar agents which can alkylate the dopamine receptor. We report here on the structure (Fig. 1) and evaluation of one agent with such potential, (-)-N-(chloroethyl)norapomorphine [(-)-NCA], and that this compound may be of significant value as a pharmacological and biochemical probe of the dopamine receptor.

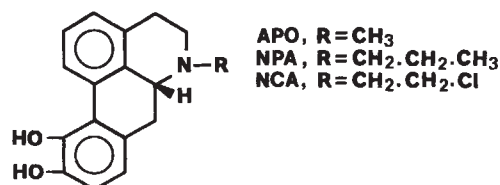


Fig. 1 The structures of apomorphine (APO), (-)-N-propylnorapomorphine (NPA) and (-)-N-(chloroethyl)norapomorphine (NCA). See ref. 8 for the synthesis of NCA.

(-)-NCA was shown to prevent the action of a reference dopamine agonist, either apomorphine or (-)-N-n-propylnorapomorphine [(-)-NPA], or to cause effects characteristic of dopamine receptor blockade, in both behavioural and biochemical assessments of action in the

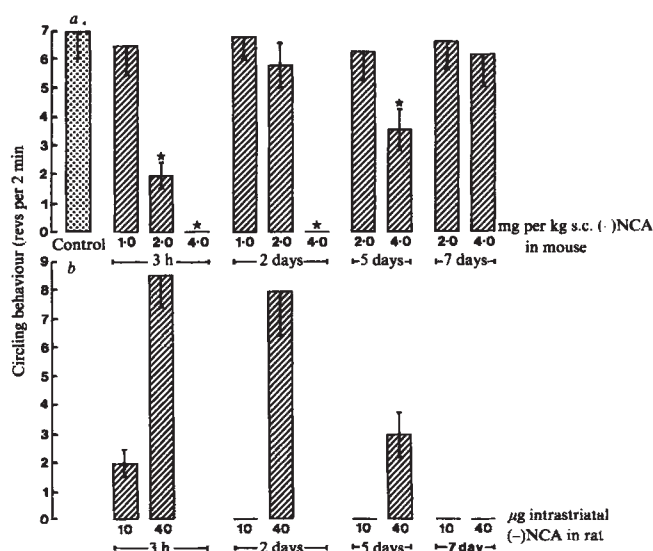


Fig. 2 *a*, Prevention by peripherally administered (-)-NCA of apomorphine induced ipsilateral circling behaviour in mice with unilateral striatal electrolesions. *b*, Induction of ipsilateral circling in rats by apomorphine following unilateral intrastriatal (-)-NCA. Circling is indicated in revs per 2 min measured in an open field and the effects of (-)-NCA within 3 h to 7 days after injection are shown. Effects on day 14 were indistinguishable from those reported for day 7. Standard stereotaxic techniques were used for the induction of striatal electrolesions in mice (male B.K.W., 25-30 g) and for implantation of guides for intracerebral injection in rats (male, Sprague-Dawley, 275-300 g) (see refs 9, 10). Control values in *a* (stippled shadings) are indicative of the circling caused in mice by 0.5 mg per kg apomorphine-HCl, (Macfarlan Smith, prepared in distilled water containing 0.1% sodium metabisulphite) given subcutaneously (s.c.) within 3 h to 7 days after treatment with vehicle for (-)-NCA.HCl (minimum quantity of *N,N*-dimethylformamide made up to volume with distilled water). In electrolesioned mice circling to apomorphine was dose-dependent, the threshold dose was 0.25 mg per kg s.c. (1-3 revs per 2 min) and the maximum was 1.0 mg per kg s.c. (10-12 revs per 2 min) (circling was not observed following appropriate solvent administration). The circling to 0.5 mg per kg s.c. apomorphine was submaximal both in electrolesioned mice receiving no further treatment or given the solvent for (-)-NCA (6-9 revs per 2 min). Circling of rats shown in *b* was caused by 0.5 mg per kg apomorphine following the unilateral intrastriatal administration of (-)-NCA, 10 and 40 µg in 4 µl. Control unlesioned untreated rats and mice showed no circling behaviour at all. *n*=6-20. Vertical bars indicate the s.e.m. values. The reduction in circling in *a* is significant to **P*<0.001 (Student's *t*-test).

dopamine-rich striatal area of the rodent brain. Thus, the ipsilateral circling behaviour induced in unilateral striatectomized mice by apomorphine, one of the most valuable models for indicating change in striatal dopamine function^{1,2}, could be abolished by the peripheral administration of (–)NCA, presumably by an action on striatal dopamine receptors in the 'intact' hemisphere (Fig. 2a). Further, it appeared that the intrastriatal injection of (–)NCA in the rat caused a blockade of dopamine receptors at the site of injection since, if the injection was made into one hemisphere only, peripherally administered apomorphine caused rats to circle ipsilateral to that side, which would again indicate a limitation of action to the 'intact' hemisphere (Fig. 2b). An important observation from each experimental situation was the persistence of the apparent dopamine inhibitory actions of (–)NCA for 2–4 days (recovery of the behavioural response possibly reflecting the resynthesis of receptors).

Confirmation that (–)NCA could indeed interact at dopamine receptors was obtained from receptor binding

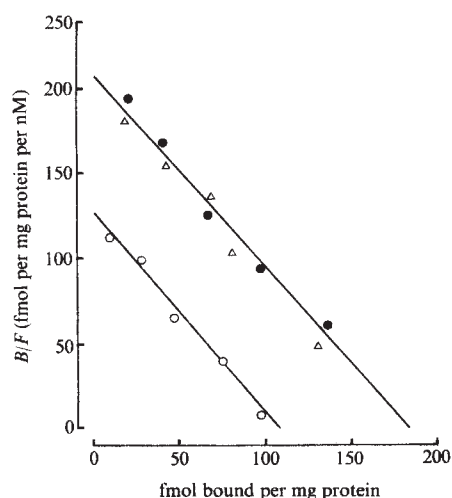


Fig. 3 Effect of intrastriatal (–)NCA on the binding of ³H-NPA to striatal tissue. Three types of striatal tissue were examined (1) that taken from control, untreated rats (●), (2) that taken from animals receiving (–)NCA (40 µg 4 µl, 1 µl per min) (○) in the left hemisphere and (3) that taken from the contralateral hemisphere to (2) and which had been subject to administration of the vehicle for (–)NCA (4 µl, 1 µl per min) (△). Binding to striatal tissue was determined on days 2, 7 and 14–16 after intracerebral injection. Only data obtained on day 2 are presented, values obtained on days 7 and 14–16 for vehicle and (–)NCA being indistinguishable from control values. Values given in the Scatchard analysis are the means of three experiments involving triplicate determinations using ³H-NPA (61 Ci mmol^{–1}, NEN) in a range of 0.0625–2.0 nM. The K_D and B_{max} values were 0.88 nM, 184 fmol per mg protein, 0.89 nM, 179 fmol per mg protein and 0.88 nM, 108 fmol per mg protein respectively for control, vehicle and (–)NCA treatments. Striata were dissected out over ice and homogenized (100 vol, w/v) in 50 mM ice cold Tris–HCl buffer (pH 7.4 at 25 °C) with a Polytron homogenizer. The homogenate was centrifuged twice (10 min, 50,000g) at 4 °C with resuspension in fresh buffer. Final resuspension (100 vol w/v) was in Tris HCl buffer (pH 7.4 at 37 °C) containing 0.1% ascorbic acid, 12.5 µM nialamide and 5 mM Na₂EDTA. Each assay tube contained 5 mg wet weight striatal tissue (equivalent to approximately 275 µg protein, measured by the method of Lowry *et al.*¹¹) in a total volume of 1.1 ml. After incubation (15 min, 37 °C) with ³H-NPA samples were rapidly filtered under vacuum over Whatman GF/B filters and rinsed rapidly with 2 × 5 ml ice cold Tris–HCl buffer: the bound radioactivity was determined. Specific binding was defined as the difference between ³H-NPA binding in the absence and presence of 10 µM ADTN and under optimal conditions accounted for approximately 75% of ³H-NPA binding.

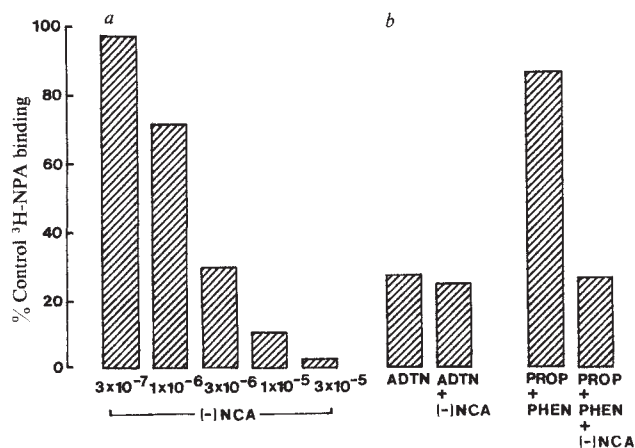


Fig. 4 Effect of (–)NCA on the binding of ³H-NPA to rat striatal homogenates. Binding is expressed in *a* as per cent of the specific binding, and in *b* as per cent of the total binding of 0.5 nM ³H-NPA (fmol per mg protein). Each point represents the mean of three experiments involving triplicate determinations. The s.e.m. values were less than 16% (calculated on original data, before conversion to percentages, for *a*, specific binding and *b*, total binding). Incubation tubes containing homogenate were preincubated for 2.5 min at 37 °C with (–)NCA (3×10^{-5} – 3×10^{-7} M), ADTN (1×10^{-5} M), ADTN + (–)NCA (3×10^{-5} M), propranolol (PROP) (1×10^{-6} M) + phentolamine (PHEN) (1×10^{-6} M) and propranolol + phentolamine + (–)NCA (3×10^{-5}) prior to the addition of 0.5 nM ³H-NPA for incubation at 37 °C for 15 min (an increase in pre-incubation times up to 30 min did not modify the effects of drug treatments). Membranes were collected by filtration as indicated in Fig. 3 legend.

studies using ³H-NPA as ligand. Subsequent to establishing the value of this ligand for the characterization of dopamine receptors, as indicated by other workers^{3,4}, it was shown that intrastriatal (–)NCA could significantly reduce ³H-NPA binding and that this biochemical change followed the behavioural effects which essentially reflected 'striatectomy' by (–)NCA. Thus, on day 2, when animals showed not only an active ipsilateral circling when challenged with apomorphine but also exhibited a resting ipsilateral asymmetry, each identical to effects one would expect from striatectomy or striatal dopamine receptor blockade, the number of binding sites for the dopamine agonist ³H-NPA was reduced by approximately 50%. As the behavioural effects of (–)NCA disappeared on days 7 and 14, so the number of sites available for the binding of ³H-NPA returned to control values (Fig. 3). That the reduction in ³H-NPA binding reflects a change in receptor numbers rather than a change in affinity for the receptor sites is indicated by the constancy of K_D values (0.88–1.12 nM) for binding to striatal tissue both from control animals and those treated for various times with (–)NCA.

The ability of (–)NCA to inhibit the binding of ³H-NPA was further established in *in vitro* experiments which facilitated an examination of the doses and conditions under which (–)NCA could inhibit dopamine mechanisms. It was shown that (–)NCA caused a 50% inhibition of ³H-NPA binding at 1.8×10^{-6} M (Fig. 4), and that this inhibition was also apparent in the presence of high concentrations of phentolamine and propranolol which could be expected to hinder occupancy of other catecholamine receptors by either ³H-NPA (see also ref. 4) or (–)NCA (it is accepted that although this indicates a dopamine involvement with the (–)NCA/³H-NPA interaction, the use of further ligands would be required to establish specificity). Further, the inhibition of 'specific' ³H-NPA binding by 2-amino-6,7-dihydroxytetralin (ADTN, for suitability of use see refs 5–7) could not be increased further by (–)NCA, indicating that the ability of (–)NCA to prevent ³H-NPA binding does not occur at

'nonspecific' sites. Finally, the prevention of ^3H -NPA binding by $(-)\text{NCA}$ (10^{-5} M) was not reversed by repeated washing.

Thus our results indicate that $(-)\text{NCA}$, when administered peripherally or intrastrially, can cause behavioural change and concomitant change at the receptor level to indicate a persistent dopamine receptor blockade.

The persistence of the blockade, and the structure of $(-)\text{NCA}$, preclude an analogy with the dopamine receptor blockade caused by typical neuroleptic agents such as haloperidol. We propose that the process by which $(-)\text{NCA}$ can inhibit dopamine receptor function may involve covalent bonding of the receptor more analogous to the action of phenoxybenzamine on the noradrenaline receptor.

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Control of X chromosome transcription by the maleless gene in *Drosophila*

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In *Drosophila*, a large group of structural genes exhibit coordinate regulation, not because they function in a common developmental pathway but because they happen to reside on the X chromosome. These genes are subjected to the regulatory mechanism of dosage compensation which insures that their phenotypic products are identical in the sex with one and in the sex with two X chromosomes. This equalization of gene products is achieved by regulating the level of transcription of both X chromosomes in females and of the single X chromosome in males¹. We report here that, reasoning that sex-specific lethal mutations may represent lesions in the processes controlling the transcription of X-linked loci, we sought and recovered several male-specific lethal mutations and noted that they affect the levels of X-linked enzyme activities in crude extracts of homozygous male larvae. Autoradiographic monitoring of RNA synthesis in larval polytene chromosomes of males homozygous for one of these mutations, *mle*^{ts}, reveals a significant reduction in the rate of X chromosome transcription.

A screen of ethyl methane sulphonate-treated major autosomes resulted in the isolation of four new male-specific lethal mutations representing three complementation groups on chromosome 2. No female-specific lethals were recovered². The mutants male-specific lethal-1 (*msl-1*) and lethal-1b (*msl-1*^b) are alleles of a gene located at position 2-53.3; male-specific lethal-2 (*msl-2*) is at position 2-9.0; the mutant maleless-temperature-sensitive (*mle*^{ts}) is a conditional allele of *mle*, a mutant previously described^{3,4} and located at 2-56.8. In homozygotes (at 25°C or at higher ambient temperatures for

Table 1 Nucleic acid synthesis in homozygous *mle*^{ts} males and females

	Sex	No. of nuclei	No. of glands	Average [X]	Average [2R]	r	b ± s.d.
RNA	Females	39	6	103	101	0.997	0.948 ± 0.034
	Males	48	9	135	188	0.995	0.761 ± 0.028
DNA	Females	31	6	417	391	0.979	1.099 ± 0.077
	Males	38	6	180	301	0.990	0.675 ± 0.029

Average [X] and average [2R]=overall average of grain counts over the X and 2R, respectively. r, Correlation coefficient of the mean grain counts for individual glands, [X] and [2R]. b, Regression coefficient of [X] on [2R].

mle^{ts}) each of these recessive mutations kills males but has no detectable effect in females.

The activities of eight enzymes were measured in crude extracts of third instar male larvae homozygous for each of the male-specific lethal mutants; controls were heterozygous male sibs and a mixture of heterozygous and homozygous mutant female sibs. Four of the enzyme activities are X-linked: glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD), fumarase (FUM) and β -hydroxy acid dehydrogenase (β -HAD); four are autosomal: α -glycerophosphate dehydrogenase (α -GPDH) and alcohol dehydrogenase (ADH) on chromosome 2, NADP-dependent isocitrate dehydrogenase (IDH-NADP) and aldehyde oxidase (AO) on chromosome 3. The results, presented in Fig. 1, show a significant reduction in G6PD, 6PGD and FUM activity in males homozygous for any one of the four mutants to about 60% of that found in controls. In contrast, the levels of all autosomal enzymes measured are not affected by the male-specific lethals. The failure of β -HAD to fit into this pattern may be attributable to several factors. This enzyme shows a peak of activity in mid-larval stages and a rapid decline as larvae approach pupation⁵. If homozygous mutant males were selected at an earlier developmental stage than control larvae, real differences in activity between these types may have been masked. β -HAD is present mainly in malpighian tubules and is almost completely absent in the fat body⁶. Extracts from homozygous mutant males in which the fat body is smaller than in controls would yield a relatively higher specific activity for the enzyme. The validity of such explanations is suggested by measurements of enzyme levels in adult male escapers homozygous for *mle*^{ts} (data not shown). These males exhibit a reduction in β -HAD activity comparable with that of other X-linked enzymes. Once again, autosomal enzyme activities are not affected by the presence of the mutant.

In *Drosophila*, the polytenic X chromosome in the salivary glands of male larvae contains half the amount of DNA^{7,8} but is as wide as the two paired X chromosomes of females. The bloated appearance of the male X is thought to result from the accumulation of some products of gene activity and to represent, therefore, a cytologically visible manifestation of dosage compensation⁹. Examination of polytene chromosome preparations revealed that the X of homozygous *mle*^{ts} male larvae was narrower and more intensely stained than the X of heterozygous males, suggesting a reduction in X chromosome function by the *mle*^{ts} mutation (Fig. 2a, b). The chromosomes of *mle*^{ts} homozygous females seem to be normal (Fig. 2c). This analysis could not be extended to males homozygous for the other male-lethal mutations as they do not have good salivary gland chromosomes.

To corroborate that the *mle*^{ts} mutation reduces the transcription of X-linked genes, we used autoradiography to monitor RNA synthesis by the polytene chromosomes of salivary glands. The results, presented in Table 1 and Fig. 3a, indicate that the rate of incorporation of ^3H -uridine by the X in *mle*^{ts} males is 80% of that in mutant females. These measurements must be corrected for the fact that a chromosome which is synapsed with a homologue emits fewer β particles capable of producing silver grains than does an

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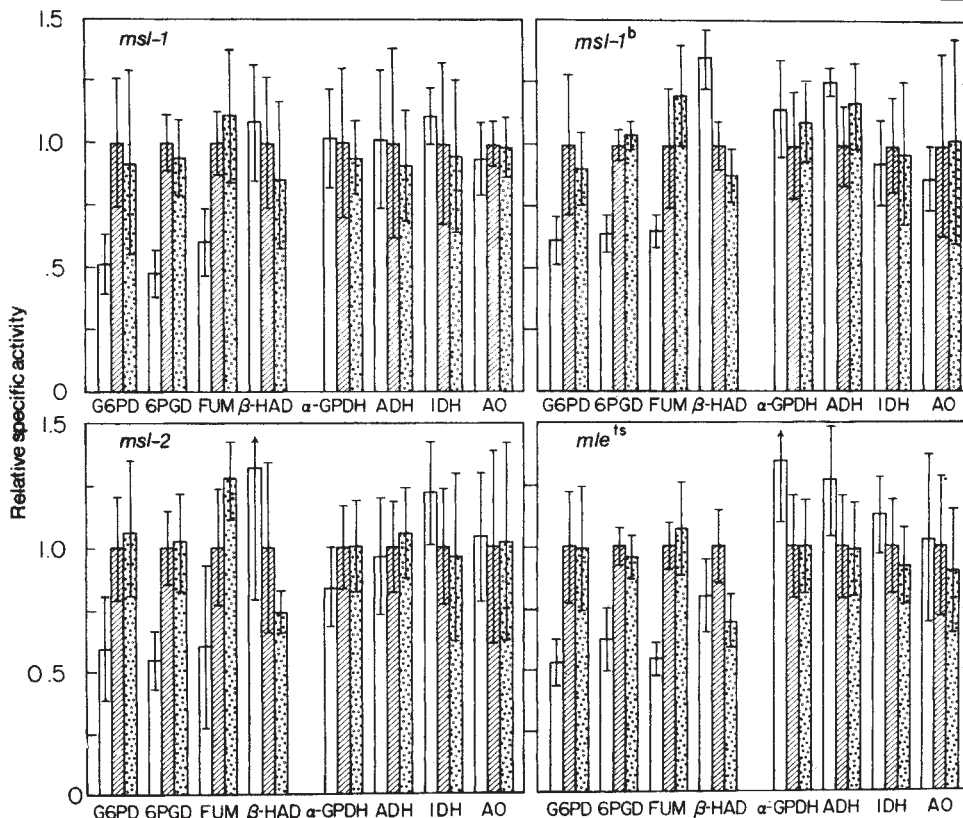


Fig. 1 Enzyme activities in mutant and control larvae. The various strains were cultured on standard cornmeal-agar-molasses-yeast medium supplemented with active dry yeast and maintained at 25 °C. Large third instar larvae were selected when they left the food and began to crawl on the side of the culture bottles. Males and females could be easily separated on the basis of gonad size. Homozygous mutant male larvae developed very slowly and crawled out of the food long after control larvae had pupated (up to 12–14 days post-hatching, as opposed to 4–5 days for control males and females²). Extracts were prepared by homogenizing third instar larvae which had crawled out of the medium at a concentration of eight individuals per ml of 0.1 M Tris, 0.27 mM EDTA and 1 mM phenylthiourea (to inhibit phenol oxidase activity) at pH 8.0. The extracts were allowed to stand on ice for 15 min and were centrifuged at 10,000g in a refrigerated centrifuge for 30 min. The clear supernatant was used as a source of enzyme in the assays. The assays for G6PD¹¹, 6PGD¹², β-HAD⁵, FUM¹³, ADH¹⁴, α-GPDH¹², IDH-NADP¹⁵ and AO¹⁶ have been published previously. Extract protein concentrations were determined using bovine serum albumin as a standard¹⁷. Enzyme activities were expressed as change in absorbance per min per mg of protein. The heterozygous males are given relative specific activity values of 1.0, and the 95% confidence limits of the means are indicated. Open columns, homozygous mutant males; hatched columns, heterozygous males; stippled columns, homozygous and heterozygous females.

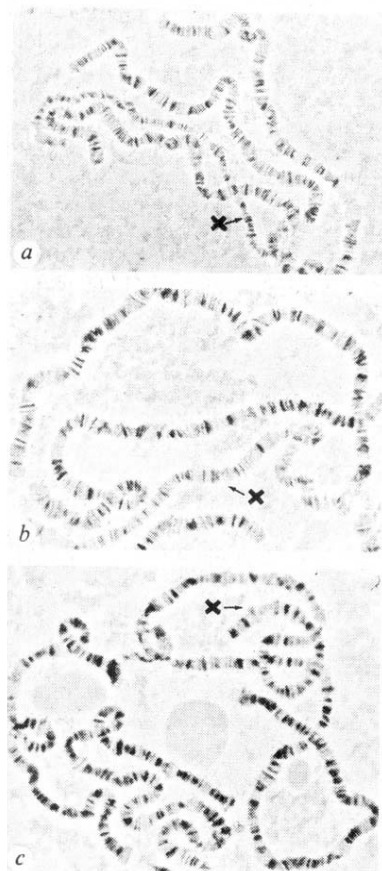


Fig. 2 Phase contrast photomicrographs of larval salivary gland chromosomes. Glands were dissected in buffered Ringer's, fixed for 5 min in 45% acetic acid and squashed. Following removal of the coverslip, they were post-fixed in ethanol:glacial acetic acid (3:1), stained with acetic carmine, dehydrated and mounted in Euparal. *a*, Homozygous *mle*^{ts} male; *b*, heterozygous male; *c*, homozygous *mle*^{ts} female.

unpaired chromosome¹⁰. This correction can be achieved by expressing RNA synthesis data in terms of the DNA content of the chromosomes determined by the same technique, that is, by autoradiography. Measurements of ³H-thymidine incorporation following chronic exposure to this labelled DNA precursor are presented in Table 1 and Fig. 3*b*. The slope of the *mle*^{ts} female measurements is not twice that of the *mle*^{ts} males, but only 1.63 times greater, a difference in magnitude consistent with our expectations based on the absorption of β particles in wild-type larvae⁸. Figure 3*c* repeats the data presented in Fig. 3*a* but with the female values corrected for the differential self-absorption of β particles by the X chromosomes in the two sexes. The slope of the line of X against autosomal activity in *mle*^{ts} females is now 1.17 (95% confidence interval: 1.12–1.21); in mutant males the slope of X against autosomal activity is 0.76 (95% confidence interval: 0.74–0.78). These results show that the rate of X chromosome RNA synthesis in *mle*^{ts} males is only 65% of that in *mle*^{ts} females. This is in striking contrast to wild type, where the rate of RNA synthesis by the single X chromosomes in males is equivalent to that of the two X chromosomes in females^{8,10}.

Our data provide complementary lines of evidence that the *mle*^{ts} mutation produces a 35–45% reduction in the transcription of X chromosome loci in mutant males. Because mutations at two other loci studied (*msl-1* and *msl-2*) similarly reduce the specific activity of X-linked enzymes but not of autosomal enzymes, it seems likely that the wild-type alleles of these loci also specify products necessary for normal levels of X chromosome transcription in males. We believe this to be the first demonstration of single mutations with a conspicuous regulatory effect on the transcription of a large segment of the *Drosophila* genome.

Of considerable interest is the particular sex-related mechanism in which these regulatory genes function. Dosage compensation is a mechanism which modulates X chromosome transcription so as to equalize, in males and females, the products of X-linked genes not involved in sex differentiation. Because dosage compensation seems to be functioning in all X chromosomes/sets of autosomes classes

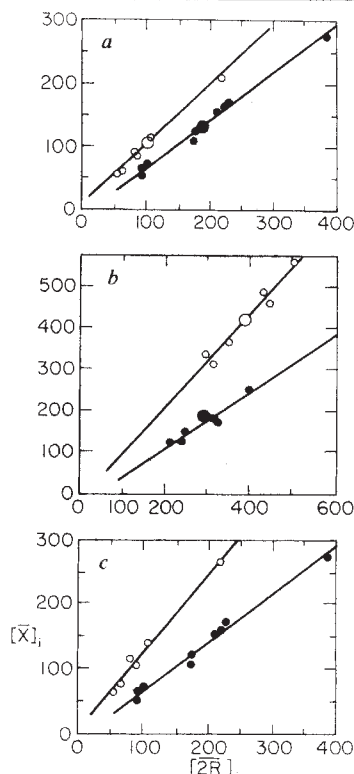


Fig. 3 Autoradiographic analysis of *mle^m* male and female larval salivary gland chromosomes. For RNA synthesis measurements, mature third instar larval salivary glands were excised and incubated in buffered Ringer's solution containing [5,6-³H]uridine (NEN, 40 Ci mmol⁻¹) at a concentration of 10 μ Ci ml⁻¹. Incorporation was stopped after 5 min by transferring the glands to 45% acetic acid. Salivary gland chromosome squashes were prepared for autoradiography by a standard procedure¹⁸. Autoradiographs were exposed for 17 days. Grains were counted over the entire euchromatic portion of the X and 2R. For chronic labelling of the DNA, first instar larvae were placed on standard *Drosophila* medium containing [5,6-³H]thymidine (NEN, 6.7 Ci mmol⁻¹) at a concentration of 20 μ Ci ml⁻¹. Mature third instar larvae were dissected in buffered Ringer's solution and salivary gland chromosomes prepared for autoradiography as above. Autoradiographs were exposed for 3 days. Grains were counted over the X and 2R. For all autoradiographs mean grain counts over the X chromosome [$\bar{X}$] and the autosome [$\bar{2R}$] were computed for every gland in which there were three or more scorable nuclei. The slope of the regression line of [$\bar{X}$] on [$\bar{2R}$] is a measure of the average increment in the number of grains over the X which accompanies a unitary increment in the number of grains over the autosome. This slope, represented by the regression coefficient *b*, is a measure of the rate of RNA synthesis by the X, or of its DNA content, relative to these parameters in the autosome. $\circ$, *mle^m/mle^m* females; $\bullet$, *mle^m/mle^m* males. The large symbols represent the overall means. *a*, ³H-uridine incorporation; *b*, ³H-thymidine incorporation; *c*, ³H-uridine incorporation, corrected for differential self-absorption of β particles by the X chromosomes in the two sexes; female values were multiplied by 2×0.675 , $1.099 = 1.23$.

and because male-specific lethal genes have no effect in females, these genes must act at a control level other than that determined by the X:A ratio. This ratio may affect the preconditions for X chromosome transcription, for example, by altering the accessibility of its chromatin. The wild-type products of male-specific lethal genes may, in turn, permit the higher levels of X chromosome transcription to occur (perhaps by modifying RNA polymerase molecules to make them more specific for X chromosome binding sites). In females, where each X is transcribed at a relatively low rate, the X:A balance would dictate the rate of transcription and the male-specific lethal gene products would not be required. In males, the X:A balance would allow high levels of X transcription which, for it to occur, would require the products of the male-lethal loci.

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Binding of adenovirus VA RNA to mRNA: a possible role in splicing?

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Most, though not all^{1,2}, of the messenger RNAs of higher cells are composed of transcripts from two or more non-contiguous DNA segments that are 'spliced' together by mechanisms which are poorly understood. There has been recent speculation that small RNA molecules may play a part in the splicing reaction, acting as templates or adaptors to stabilize the appropriate conformation of a precursor RNA³⁻⁶. Adenovirus-2 codes for two low molecular weight RNAs, the virus-associated (VA) RNAs I and II, major and minor species, respectively⁷. These RNAs are about 160 nucleotides long and have both been sequenced⁸. They originate from closely spaced genes which are transcribed by RNA polymerase III⁹, but have not been definitively associated with any function. We have shown previously⁷ that a fraction of the VA RNA of infected cells is complexed with high molecular weight RNA in a denaturation-sensitive fashion. Results presented here show that the VA RNAs bind to unfractionated late virus mRNA and to a cloned copy of a single mRNA species, but not to corresponding cloned segments of viral genomic DNA. It is suggested that VA RNA may act as a template in the splicing reaction.

To assay the binding, ³²P-labelled VA RNA is incubated in conditions used for R-loop formation¹⁰ with unfractionated RNA isolated from the cytoplasm of adenovirus-2-infected HeLa cells. The RNA is precipitated from the reaction, redissolved and passed over a column of oligo(dT)-cellulose to trap polyadenylated RNA together with any associated molecules. The column is washed and the poly(A)⁺ RNA is eluted with a buffer of low ionic strength. Formation of complex is detectable after 1 min of incubation, reaches a maximum at 30 min and remains stable for at least 5 h. Elution profiles obtained with a reaction that was cycled three times over oligo(dT)-cellulose are shown in Fig. 1a-c. About 70% of the radioactivity in the poly(A)⁺ fraction chromatographs with the poly(A)⁺ fraction through second and third cycles of chromatography. Only trace amounts of VA RNA that had been incubated in the absence of cytoplasmic RNA appeared in the poly(A)⁺ fraction, and none of this nonspecifically bound and eluted ³²P-VA RNA stuck to the column when re-applied, validating the assay and verifying that adherence to the column requires an interaction between the VA RNA and component(s) of the infected cell RNA. Other control experiments showed that preselected poly(A)⁺ RNA can bind

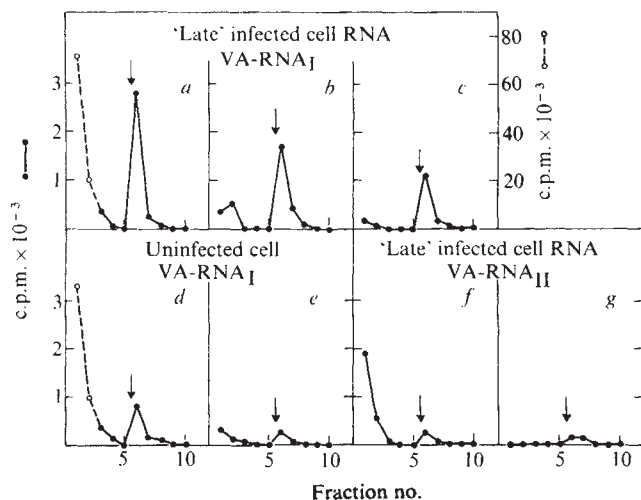


Fig. 1 Binding of VA RNA to polyadenylated RNA. ^{32}P -labelled VA RNA, isolated by two-dimensional gel electrophoresis⁷, was incubated with cytoplasmic RNA¹⁴ at 50°C for 3 h in 100- μl reactions containing 70% formamide, 0.4 M NaCl and 0.01 M PIPES buffer (pH 6.8). Reactions were terminated by chilling and dilution. The RNA was precipitated, dissolved in TE (10 mM Tris-HCl, pH 7.4, 1 mM EDTA), and applied to a 0.3-ml column of oligo(dT)-cellulose in 1 ml of TE containing 0.5 M NaCl. The flow through was re-applied three or four times to obtain maximal binding of poly(A)⁺ RNA and the column was washed with four 1-ml aliquots of the loading buffer. Polyadenylated RNA was eluted with five 1-ml aliquots of TE buffer. The radioactivity in each fraction was determined by Cerenkov counting. For recycling of the poly(A)⁺ material (b, c, e, g), appropriate fractions were pooled from the experiments of a, b, d and f, respectively, made 0.5 M with NaCl, and re-applied to the column as described above. Reaction a contained 100 μg of cytoplasmic RNA isolated 27 h after adenovirus-2 infection of HeLa cells and 95,000 c.p.m. of VA RNA_I; fractions 6 and 7 were recycled (b), and fractions 6 and 7 were recycled again (c). Reaction d contained 56 μg uninfected HeLa cell cytoplasmic RNA and 86,000 c.p.m. of VA RNA_I; fractions 6–8 were recycled (e). Reaction f contained 100 μg infected cell RNA and 3,000 c.p.m. of VA RNA_{II}; fractions 6 and 7 were recycled (g). The arrows mark the beginning of the elution of polyadenylated RNA.

VA RNA, and that the product is sensitive to ribonuclease; these experiments strongly indicate that the VA RNA is indeed binding to mRNA.

The specificity of the reaction is the subject of Fig. 1d–g. A small amount of radioactive VA RNA bound to and eluted from the column with the poly(A)⁺ fraction of uninfected cell RNA, but little of this re-bound in a second cycle (Fig. 1d,e). Bearing in mind that the radioactivity of the VA RNA is heavily diluted by the presence of VA RNA in the infected cell RNA but not in the uninfected cell RNA, it seems that the latter binds VA RNA very much less effectively than does the infected cell RNA. Figure 1f,g shows the only experiment described here which used the minor VA RNA_{II} species. Although only limited amounts of radioactivity were available, it is apparent that this RNA was also bound to and eluted from the column with late poly(A)⁺ RNA and that most of the counts in this fraction chromatographed with the poly(A)⁺ fraction when re-cycled. Thus, the interaction between poly(A)⁺ RNA and VA RNA is specific for infected cell RNA but not for the species of VA RNA.

To explore the nature of the complex, material isolated after one passage over oligo(dT)-cellulose was subjected to electrophoresis in an agarose gel in non-denaturing conditions. Figure 2, lane c, shows that most of the radioactivity contained in the complex penetrated the gel only slightly and that a small fraction ran with the mobility of free VA RNA (lane a). Much (if not all) of the free VA RNA in lane c presumably represents the portion which fails to

chromatograph with the poly(A)⁺ fraction on re-cycling over oligo(dT)-cellulose. Heating to 100°C released all the VA RNA to run with the mobility of the marker (Fig. 2, lane e), showing that it is present in a substantially intact form as part of a thermally unstable complex. Moreover, the complex was resistant to the detergent SDS (Fig. 2, lane d), suggesting that protein contaminants are probably not involved in its stabilization. These data indicate that the complex is likely to be held together by base pairing forces but yields no information on the nature of the other component because the mobility of the complex, being much less than that of known adenovirus mRNAs, implies either an outspread conformation or a multimeric nature, and in any case does not permit identification of the other protagonist.

To study the viral RNA involvement, a cloned DNA copy¹¹ of a late viral mRNA, coding for the fibre protein, has been used. In the plasmid pJAW43, the DNA sequences inserted into the pBR322 vector include the first, second and third segments of the tripartite leader common to most late adenovirus mRNAs^{12,13}, the fourth leader specific for some forms of fibre mRNA^{13,14}, and about half of the sequences corresponding to the main body of the fibre mRNA and representing the N-terminal portion of the protein. The results in Table 1, expt 1, indicate that VA RNA hybridizes to nitrocellulose filters carrying this recombinant plasmid but not to filters carrying pBR322 DNA alone. Ribonuclease T₁ fingerprints⁷ of the material thermally eluted from the filter, or indeed from the complex with poly(A)⁺ RNA, confirmed that the bound material is indeed VA RNA_I and not a contaminant. Experiment 2 (Table 1) shows that VA RNA does not bind to unrelated viral DNA sequences, such as a cloned cDNA copy of the unspliced polypeptide IX mRNA², even if they are inserted by the same A-T tailing method used in constructing pJAW43 (as is the case with pJAW1).

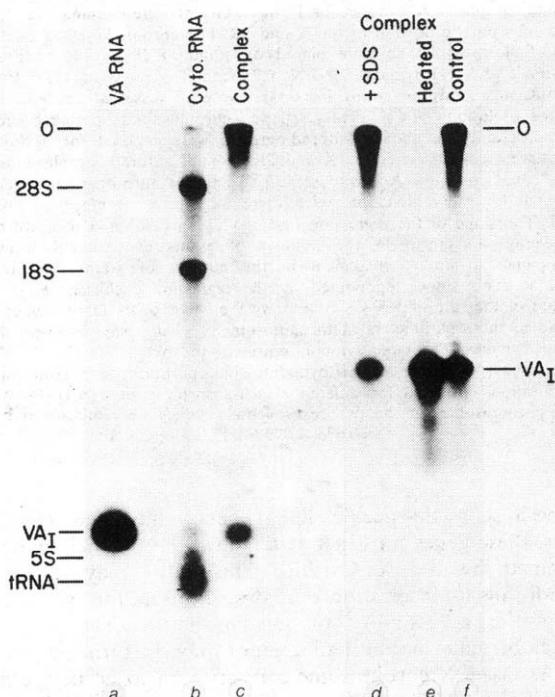


Fig. 2 Analysis of VA RNA:mRNA complex by agarose gel electrophoresis. Complex between ^{32}P -labelled VA RNA_I and polyadenylated late infected cell RNA was isolated as in Fig. 1b,c, precipitated, and dissolved in TE buffer. Aliquots were subjected to electrophoresis through 2% agarose gels in TBE buffer (89 mM Tris, 89 mM boric acid, 1 mM EDTA), and the gels were dried on DEAE-paper before autoradiography. Lane c, untreated complex; lane d, complex incubated for 15 min at room temperature with 0.1% SDS; lane e, complex heated to 100°C for 1 min; lanes a, b, marker tracks with ^{32}P -labelled VA RNA_I and cytoplasmic RNA from infected cells, respectively.

Table 1 Hybridization of VA RNA with immobilized fragments of adenovirus DNA

Plasmid	Viral sequences	³² P-VA RNA bound (c.p.m.)		
		Expt 1	Expt 2	Expt 3
None	—	114	396	113
pBR322	—	177	—	—
pJAW43	Fibre mRNA-cDNA	45,321	55,698	22,611
pJAW1	Protein IX mRNA-cDNA	—	361	—
pAd21	31.5–37.3 m.u.	—	117	—
pAd5	73.6–79.9 m.u.	—	71	—
pAd33	80.6–89.5 m.u.	—	108	—
pAd20	89.5–97.3 m.u.	—	619	—
pBa1E	14.7–21.5 m.u.	—	—	80
pBa1D	21.5–28.5 m.u.	—	—	60

Plasmid DNA was linearized by cleavage with a restriction enzyme, extracted with phenol and with chloroform, alcohol precipitated, denatured by boiling in water and applied to 3-mm squares of nitrocellulose paper¹⁷. Pairs of filters were incubated with ³²P-VA RNA_i for 3 h at 50°C in 50 µl of solution containing 70% formamide, 0.4 NaCl, 0.01 M PIPES buffer (pH 6.8) and 0.1 mg ml⁻¹ calf liver tRNA. The free liquid was removed and the filters were washed 15 times in 1 ml of 1×SSC (0.15 M NaCl, 0.015 M Na citrate) with 0.5% SDS by vigorous vortexing followed by aspiration. Filters were then washed three times with 1 ml of TE (10 mM Tris-HCl pH 7.4, 1 mM EDTA) containing 0.15 M NaCl, and three times with 1 ml of TE. Radioactivity was determined by Cerenkov counting. Incubations contained 1.4, 0.5 and 0.3 × 10⁶ c.p.m. of VA RNA in expts 1, 2 and 3, respectively. In expt 1, each filter carried 10 µg pJAW43 DNA or 4 µg pBR322 DNA; in expts 2 and 3, each filter carried recombinant DNA equivalent to 10 µg of adenovirus-2 DNA. m.u., Map units.

Furthermore, VA RNA fails to anneal with cloned segments of the virus genome containing various portions of the fibre mRNA sequence—the first and second leaders (16.5–16.6 and 19.5–19.7 map units), the third leader (26.5–26.8 map units), the fourth leader (78.6–79.1 map units) and the mRNA body (86.3–91.2 map units)¹³. Thus, the binding must depend on a cooperative interaction between VA RNA and non-contiguous segments of viral DNA which are brought together in the pJAW43 plasmid. Presumably, when the individual components of the sequence which binds VA RNA are separated, the binding is too weak to be detected by the assay used: the low melting temperature of the hybrid, 55°C in 0.3 M salt, is consistent with a structure composed of one or more short or imperfect duplexes. Experiments to pinpoint the interactions are in progress.

These results are open to more than one interpretation. One possibility is that VA RNA binds to spliced mRNA(s) and is involved in some subsequent process, such as transport from the nucleus into the cytoplasm or protein synthesis (although no effect of VA RNA binding on cell-free translation of viral mRNA has been detected). An alternative and attractive hypothesis is that VA RNA plays the part of a template in the splicing reaction, bridging two conserved regions which flank intervening sequences within a precursor RNA. In this way, the 5' and 3' borders of the impending splice point could be juxtaposed in preparation for the cleavage and ligation reactions. The existence of two VA RNA species, synthesized at different rates during the course of the infections⁹, might allow temporal regulation of the production of late viral mRNAs from their common precursor¹⁵. One can also speculate that small nuclear RNAs of uninfected cells could serve a similar function in the generation of cellular mRNAs^{6,16}.

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F plasmid provides a function that promotes *recA*-independent site-specific fusions of pSC101 replicon

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Recently, it has been reported that the tetracycline (Tc) resistance plasmid pSC101 (ref. 1) can undergo integrative recombination (that is, fusion) with a plasmid or bacteriophage genome that lacks extensive DNA sequence homology with pSC101. Such fusion of pSC101 with a second replicon can occur in the absence of the bacterial *recA* gene function, and seems to involve DNA sequences on pSC101 that closely resemble the inverted repeat termini of the transposable genetic element, Tn3 (refs 2, 3). In both of the observed instances of replicon fusion involving pSC101, the fertility plasmid F was present in the bacterial cell; in one case an autonomously replicating F plasmid was used to support the infection of the male-specific bacteriophage ϕ 1 (ref. 2), whereas in the other F was integrated into the chromosome of an Hfr bacterial strain being used to study mobilization of a non-conjugative plasmid⁴. However, in many studies with the pSC101 plasmid carried out in the absence of F in our laboratory and elsewhere, pSC101 has not been observed to undergo fusion to a second concurrently present replicon. These observations suggested to us that the F plasmid might carry a function that enables pSC101 to undergo replicon fusion. We report here results indicating that the F plasmid does, in fact, provide a *trans-acting* function that promotes *recA*-independent site-specific recombination by pSC101, and that the function carried by F is located in the segment of the plasmid that includes the *tra* genes and the $\gamma\delta$ sequence.

The pPM103 plasmid⁵, which is a temperature-sensitive (*ts*) mutant of pSC101, and the ColE1 plasmid were introduced independently by transformation into a *recA56* derivative of *Escherichia coli* strain C600 (PM191, constructed in our laboratory by P. Meacock). F⁺Km, an F plasmid derivative carrying a kanamycin (Km) resistance gene (from R. Curtiss III), was introduced by conjugation⁶ into a clone carrying the other two plasmids. F⁺Km and F⁻ strains were grown overnight at 30°C in the absence of Tc and then plated at 42°C in the presence of Tc (see Table 1). A variation of the selected translocation procedure⁷ was used to identify clones in which fusion of pPM103 to another replicon had occurred; as replication of pPM103 does not take place at 42°C (ref. 5), isolation of Tc-resistant cells following growth at this temperature implies that either recombination of pPM103 with a non-*ts* replication system or reversion of its *ts* mutation has occurred.

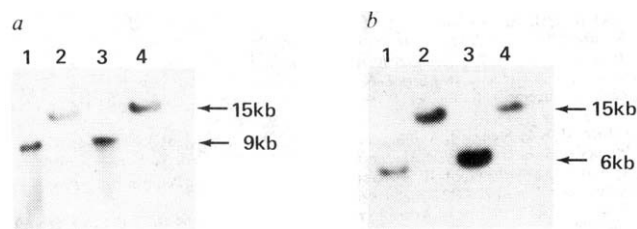


Fig. 1 Plasmid DNA isolated from an F^+ or F^- clone carrying the pPM103 and ColE1 plasmids was treated with *Bam*HI (or *Eco*RI) endonuclease, subjected to electrophoresis on agarose gels²⁵ and analysed by hybridization on nitrocellulose filters¹⁶. The ³²P-labelled probes were: *a*, pSC101; *b*, ColE1. A molecular size standard consisting of *Eco*RI-cleaved pRR12⁹ was used to indicate fragment length. *a*, Lane 1, pSC101; lane 2, pSC134 (an *in vitro* recombinant of ColE1 and pSC101 linked at the *Eco*RI site of each plasmid²⁶); lane 3, plasmid DNA from a Tc^R colony of PM191 (pPM103, ColE1) grown at 42°C; lane 4, plasmid DNA from a Tc^R similarly grown colony of PM191 (F^+ Km, pPM103, ColE1). All DNAs were digested with endonuclease *Bam*HI. *b*, Lane 1, ColE1; lanes 2, 3, 4 are the same as *a*. Lanes 1 and 3, DNA digested with endonuclease *Eco*RI; lanes 2 and 4, DNA digested with endonuclease *Bam*HI.

Table 1 shows the effect of the F^+ Km plasmid in promoting fusion of pPM103 to a concurrently present replicon. As can be seen, the presence of an F^+ Km resulted in an 80-fold increase in the fraction of cells that retain Tc resistance after growth at 42°C (expts 1 and 2); all but two of 21 F^+ Km clones examined contained a 15-kilobase plasmid equal in size to the sum of pPM103 (9 kilobases) and ColE1 (6 kilobases). The remaining two colonies growing at 42°C in the presence of Tc contained two small plasmids the size of ColE1 and pPM103 and presumably represent instances of reversion of the temperature-sensitive mutation of pPM103. No instance of fusion of pPM103 to F itself or to the bacterial chromosome was observed when the multicopy ColE1 plasmid was also present. However, insertion of pPM103 into F in Tc-resistant clones occurred at approximately the same frequency as reversion of the *ts* mutation following growth at 42°C in the absence of ColE1 (our unpublished data). In bacteria lacking F, no co-integrate plasmid was observed in 17 colonies examined (expt 1); 16 of the 17 colonies contained two small plasmids the size of pPM103 and ColE1; the remaining colony had a single plasmid the size of pPM103. When ColE1 was replaced by the pACYC177 (ref. 4) (Km^R, Ap^R) or pTU4 (ref. 8) (Cm^R) plasmids (both of which use the p15A plasmid replicon) or the pDPT234 plasmid⁹ (an Sp^R plasmid carrying replication functions of an NR1 plasmid copy number mutant), in the above experiments, analogous results were observed (expts 4 and 5 and unpublished data). However, the increased frequency of rescue of pPM103 with pDPT234 (expts 4, 5) was 10–30-fold higher than with ColE1 (expts 1, 2).

The R6-5 antibiotic-resistance plasmid¹⁰, which is largely homologous with F in the fertility region¹¹ but carries repressed *tra* genes, does not promote fusion of the pPM103 plasmid (expt 3); however, a closely related plasmid (R100.1)¹², which is de-repressed and thus expresses *tra* functions at a high level, does promote replicon fusion (expt 6), and this occurred at a frequency two orders of magnitude higher than was seen with F. In contrast to the results with F, R100.1-promoted fusion involved insertion of pPM103 into the R100.1 plasmid itself even in the presence of ColE1; preliminary evidence indicates that such insertion occurs preferentially at a specific location within R100.1. An F^- plasmid mutant deleted for almost half of the molecule including the *tra* gene segment (JC7247, FΔ446 (ref. 13), expt 10) fails to promote replicon fusion. Plasmids containing individually cloned *Eco*RI fragments from the *tra* gene region¹⁴ of the F plasmid also do not promote replicon fusion (expt 7); such fusion is accomplished, however, by F^- plasmid derivatives that contain single mutations in *tra* genes A, C, D, E, J of I (ref. 15 and unpublished data). Thus the *tra* genes

themselves may not be involved in 5 promoted replicon fusion. The $\gamma\delta$ sequence, a transposable element known to have Tn3 light termini similar to those on the pSC101 plasmid²⁷ is present on the F plasmid segments that promote replicon fusion, and may very well be implicated in the process.

Plasmid DNA was isolated from representative F^+ Km or F^- ColE1-containing Tc-resistant clones grown at 42°C and digested with the *Bam*HI endonuclease, which cleaves pPM103 (that is, pSC101) DNA but not ColE1, or *Eco*RI endonuclease which cleaves ColE1 once. The DNA was then subjected to electrophoresis on agarose gel and analysed by hybridization¹⁶ with ³²P-labelled pSC101 or ColE1 DNA (Fig. 1). As shown in Fig. 1, the 15-kilobase DNA species present in F^+ Km cells is a composite plasmid that hybridizes with both pSC101 and ColE1 DNA. In contrast, the two plasmids remain as separate replicons in bacteria that lack F.

Restriction endonuclease/agarose gel analysis of fusion plasmids from a series of F^+ Km clones indicates that the site of recombination is unique with respect to the pPM103 plasmid, but that it seems to be nonspecific with regard to the second small plasmid replicon (in this instance, three randomly selected clones of pDPT234; Fig. 2). Analysis of DNA digested by the *Hinc*II endonuclease, which cleaves pPM103 several times but the pDPT234 plasmid only once, shows that the same *Hinc*II fragment (fragment C) of pPM103 is lacking in all the recombinants, indicating that the junction site on pPM103 occurs within this fragment in each of these fusions. Digestion of the 15-kilobase co-integrate plasmid with the *Mbo*II endonuclease resulted in analogous findings (*Mbo*II fragment 'F' was missing), and permitted more precise localization of the pPM103 recombination site on a pSC101 endonuclease fragmentation map constructed in our laboratory by C.-P.D. Tu (unpublished data). That map shows that the *Mbo*II F fragment and the *Hinc*II C fragment overlap at a position that corresponds, within the limits of resolution by such analysis, to the site at which pSC101 was shown previously to integrate into bacteriophage ϕ 1 DNA². In contrast to the unique position of the junction with respect to

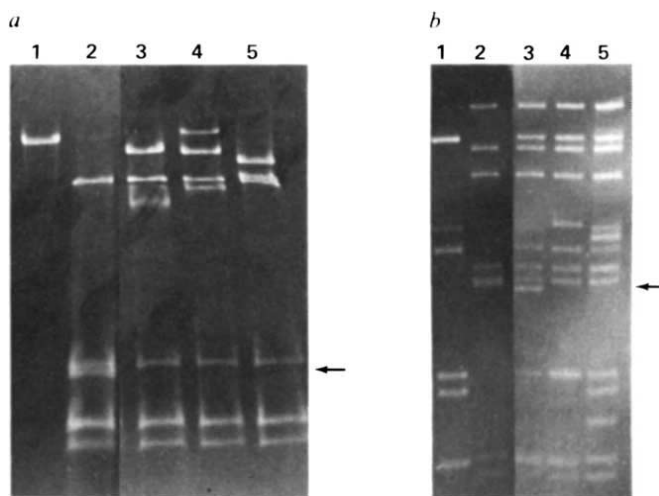


Fig. 2 *a*, Agarose gel analysis of pPM103/pDPT234 fusion plasmids. DNA samples from three separate fusion plasmids and DNA of pSC101 and pDPT234 was isolated as described²², cleaved with endonuclease *Hinc*II and analysed by gel electrophoresis²⁵. Samples were run in 1% agarose gels, Tris-borate buffer, pH 8.3, for 3 h at 150 V. pCM431, 432, 433 are 14-kilobase (kb) pPM103/pDPT234 fusion plasmids obtained in separate experiments. The pDPT234 plasmid is cleaved once by *Hinc*II endonuclease; the C band of pPM103 (arrow) is absent in all the fusion plasmids and new bands are formed. Lane 1, pDPT234; lane 2, pSC101; lane 3, pCM431; lane 4, pCM 432; lane 5, pCM433. *b*, The same DNA samples were analysed in 1.2% agarose gels run in Tris-borate for 2.5 h following digestion with endonuclease *Mbo*II. *Mbo*II band F of pPM103 (arrow) is missing in all of the independently isolated recombinants, indicating that the pPM103/pDPT234 joint is located at the same site in each of these plasmids. A different pDPT234 band is missing in each fusion plasmid, indicating that the junction with pPM103 occurs at different pDPT234 locations.

Table 1 Effect of F'Km plasmid in promoting fusion of pPM103

Bacterial strain and plasmids	Tc ^R Colonies at 42 °C/Total colonies at 42 °C	Plasmid DNA isolated from Tc ^R colonies grown at 42 °C
PM191 (pPM103, ColE1)	1.5×10^{-5}	16/17 two plasmids (9, 6) 1/17 one plasmid (9)
PM191 (F'Km, pPM103, ColE1)	1.2×10^{-3}	19/21 one plasmid (15) 2/21 two plasmids (9, 6)
PM191 (R6-5, pPM103, ColE1)	1.5×10^{-6}	10/11 two plasmids (9, 6) 1/11 one plasmid (9)
PM191 (pPM103, pDPT234)	2.5×10^{-8}	3/3 two plasmids (9, 5)
PM191 (F'Km, pPM103, pDPT234)	3.0×10^{-5}	3/3 one plasmid (14)
PM191 (R100.1, pPM103, ColE1)	1.0×10^{-1}	4/4 two plasmids (89, 6†)
PM191 (pCM1891*, pPM103, ColE1)	2.5×10^{-7}	2/2 two plasmids (9, 6)
JC1569 (pPM103, pDPT234)	9.0×10^{-8}	4/4 two plasmids (9, 5)
JC1569 (F'Km, pPM103, pDPT234)	5.0×10^{-5}	4/4 one plasmid (14)
JC1569 (F 446, pPM103, pDPT234)	4.0×10^{-7}	4/4 two plasmids (9, 5)

Single colony isolates of the *recA56* *E. coli* strain PM191 carrying the plasmids shown were grown overnight in L-broth at 30 °C in the absence of selection. The cells were diluted appropriately and plated at 42 °C with or without Tc. The ratio of Tc-resistant to total colonies after overnight growth at 42 °C is shown. Plasmid DNA isolated from cultures²² was examined by agarose electrophoresis to determine the nature and size of plasmids present. The pPM103 plasmid, a temperature-sensitive replication mutant of pSC101⁵, the ColE1 plasmid and R6-5 were introduced into bacteria by transformation²³. The F'Km plasmid was transferred by conjugation from *E. coli* strain χ 916 into PM191 (pPM103, ColE1), as was R100.1. The FΔ446 plasmid, which has a deletion of the *tra* region of F leaving the sequence between 16 and 58 min (ref. 14), was introduced into the *recA*⁻ strain JC1569²⁴ by conjugation. The plasmid pDPT234⁹ was also introduced by transformation and used as the recipient in some cases. Numbers in parentheses indicate the size of the plasmids in kilobases.

*pCM1891 = pACYC189⁴ with *Eco*RI fragment 1¹⁴ cloned into the *Eco*RI site. Similar results were obtained with *Eco*RI fragments 2, 3, 5, 6, 15.

†The very large plasmid, a composite of the R100.1 and pPM103, was found to have the pPM103 inserted into the same region in each of the isolates as determined by digestion of plasmid DNA with endonucleases *Eco*RI, *Bam*HI or *Sal*I.

pSC101, the fragment of pDPT234 that was involved in the recombinational event differed for each fusion plasmid.

Recently, it has been shown that the Tn3 transposon, which has inverted repeat termini similar to the sequences involved in site-specific recombination of the pSC101 plasmid², encodes a protein of molecular weight 100,000 essential for transposition of this element^{17,18}. We therefore tested the ability of the Tn3 transposon located on the bacterial chromosome to promote fusion of pPM103 with ColE1 or with a ColE1:Tn3 plasmid (that is, pACYC199) as a recipient for the pPM103. Our results indicate that the Tn3 transposon does not promote simple replicon fusion of the type promoted by F, but suggest that a more complex interaction of the Tn3-like sequence on pPM103 with the Tn3 termini may occur, resulting in replicon fusions involving the Tn3 transposon.

The findings reported here indicate that the *trans*-acting function provided by F and mapping in the region of the *tra* genes enables the pSC101 plasmid derivative pPM103 to undergo site-specific replicon fusion at a location where Tn3-like inverted repeat sequences have been identified previously. Current models for transposition¹⁹⁻²¹ propose that replicon fusion is an intermediate step in the transposition process. If this proposal is correct, it seems that the function provided by F is at least capable of carrying transposition through the intermediate step by accomplishing replicon fusions of pSC101 and its pPM103 derivative.

The ability of F to promote fusion of a replicon that carries inverted repeat sequences but is unable by itself to undergo site-specific recombination may be of some importance in the structural evolution of extrachromosomal genetic elements. As Tn3-like inverted repeat sequences have also been identified on at least one other small plasmid replicon (pTU4)⁸, these

findings suggest that the phenomenon we have observed may be an example of a more widespread occurrence.

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Yeast mitochondrial tRNA^{Trp} can recognize the nonsense codon UGA

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DNA sequence analysis of mitochondrial genes that code for some mitochondrial proteins has suggested that the opal terminator, UGA, is used as a sense codon in mitochondria¹⁻⁴. The complete sequences of both the yeast^{2,4} and human³ genes coding for cytochrome oxidase subunit II contain UGA codons in the reading frame. When the protein sequences predicted by these DNA sequences are compared with the known protein sequence of bovine mitochondrial cytochrome oxidase subunit II, there are regions of homology, in which UGA codons correspond to tryptophan residues. Therefore it has been suggested that UGA specifies tryptophan in the mitochondrial code. We have isolated a yeast mitochondrial tRNA^{Trp} and used it to locate the mitochondrial tRNA^{Trp} gene in pBR322-mitochondrial DNA recombinants. DNA sequence analysis of this gene revealed that the mitochondrial tRNA^{Trp} anticodon is 5'UCA3'. Because there is a U in the wobble position, this tRNA can recognize and insert tryptophan into a growing polypeptide chain in response to the nonsense codon UGA.

To determine if the mitochondrial tryptophan tRNA of yeast has a structure compatible with the hypothesis that UGA specifies tryptophan it is necessary to determine the primary sequence of the tRNA itself or of the tRNA gene.

Table 1 Charging of individual tRNAs with ^3H -tryptophan

tRNA	C.p.m. incorporated
I	735
II	44,057
III	0
IV	489
V	0
VI	0

tRNAs were charged with mitochondrial synthetase in a reaction containing 100 mM Tris, 15 mM MgCl_2 , 6 mM ATP, 0.2 mM dithiothreitol and 5 μCi ^3H -tryptophan (11.7 Ci mmol^{-1}). Trichloroacetic acid-precipitable counts were determined and blank values have been subtracted.

absence of sequence data, one can consider several possibilities that might alter the codon recognition pattern of a single mitochondrial tRNA^{Trp} so that both UGA and UGG can be read. First, if the anticodon is CCA, one or more modified bases either in the anticodon loop or elsewhere might alter codon recognition to include UGA. We are not aware of a specific precedent for this possibility. Second, a primary sequence which dictates an unusual tertiary structure for the yeast mitochondrial tRNA^{Trp} might enable it to read both

UGG and UGA. The UGA suppressor strain of *E. coli* that inserts tryptophan in response to UGA does retain a CCA anticodon but differs from the wild-type in a single base change in the D stem¹⁰. Finally, the anticodon of the mitochondrial tRNA^{Trp} might be different from other wild-type tRNAs^{Trp} so that following wobble rules, both UGA and UGG can be read. Our sequencing data are consistent with that last possibility, so that the presence of UGA in the reading frame of mitochondrial protein genes is accommodated by a tRNA^{Trp} that can recognize UGA. Unless there is a modification in the anticodon that prevents G-U wobble, this tRNA^{Trp} should also recognize the tryptophan codon UGG.

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Retinoid prevents transformation of cultured mammary glands by procarcinogens but not by many activated carcinogens

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Specific hormones^{1,2} in concert with epidermal growth factor³ induce the mouse mammary gland in serum-free whole organ culture to undergo two physiological cycles³, each consisting of lobuloalveolar development, differentiation and involution (regression). In addition, chemical carcinogens cause an epithelial transformation, which is operationally defined by the presence of nodule-like alveolar lesions that have escaped the hormonal controls of lobuloalveolar development^{4,9}. The transformed mammary glands are also dysplastic, metaplastic^{4,5} and oncogenic⁶. Chemically analogous non-carcinogens have little or no transforming activity^{4,8}. Furthermore, transformation by the carcinogen 7,12-dimethylbenz[*a*]anthracene (DMBA) is prevented, suppressed and apparently reversed by retinoid⁵ (chemical related to vitamin A). The retinoid acts anti-promotionally, that is, it apparently blocks the transformation process at a stage after the interaction of carcinogen with critical cellular target(s). We report here that the ability of retinoid to prevent mammary gland transformation anti-promotionally is largely limited to carcinogens (procarcinogens) that require metabolic activation to become reactive. Mammary gland transformations caused by low concentrations of procarcinogens are blocked by the retinoid 2-retinylidene-5,5-dimethyl-1,3-cyclohexanedione (retinylidene dimedone). In contrast, transformations by five activated carcinogens and by high concentrations of procarcinogens are not prevented. The present findings suggest important limitations of retinoids in the prevention of epithelial transformation by chemical carcinogens.

Whole mammary glands of BALB/c mice were cultured for 10 days in serum-free development medium containing insulin, prolactin, aldosterone and hydrocortisone (Fig. 1 legend)^{4,5,10}. By day 10, the glands possess fully developed lobuloalveoli^{2,4} and casein¹¹. During days 3–4 only, the cultured glands were treated with solvent containing or lacking carcinogen, and after development were maintained for 14 days (days 10–24) in

serum-free regression medium (insulin being the only added hormone). The lobuloalveoli of untransformed glands regressed completely. Transformed glands (defined in Table 1) were dysplastic, metaplastic^{4,5}, and contained one or more morphologically developed lobuloalveoli (nodule-like alveolar lesions)^{4–9}. The ability of retinoids to prevent transformation was examined by their inclusion in the development medium at different periods (Fig. 1).

Retinylidene dimedone failed to prevent transformation when administered before (days 0–3) the carcinogens (days 3–4) (Fig. 1, protocol I). This failure occurred with all the tested procarcinogens, benzo[*a*]pyrene (BP) and *N*-2-fluorenylacetylamine (FAA), and activated carcinogens, benzo[*a*]pyrene *trans*-7,8-dihydrodiol,9,10 epoxy (*anti*) (BP-diol epoxide) and *N*-acetoxy-*N*-2-fluorenylacetylamine (*N*-AcO-FAA) (Table 1). These findings agree with our results with the procarcinogen, DMBA⁵. The retinoid thus did not pre-adapt the mammary glands to block transformation by the procarcinogens or activated carcinogens. Retinylidene dimedone also failed to prevent transformation when present concurrently (days 3–4) with the above procarcinogens and activated carcinogens (Fig. 1, protocol II, and Table 1), as with DMBA⁵. The retinoid thus did not act as an anti-initiator.

Retinylidene dimedone did anti-promotionally inhibit transformations by the procarcinogens (Fig. 1, protocol IIIa). Treatment with the retinoid during days 4–10, following exposure to procarcinogens at days 3–4, blocked transformation by BP up to 100% ($P < 0.03$), and by FAA up to 61% ($P = 0.02$) (Table 2). The procarcinogen, DMBA, acted similarly⁵. The retinoid at 10^{-7} M was effective against 10-fold molar excesses of BP and FAA. However, 100-fold excesses of these procarcinogens overcame the retinoid, resulting in no significant prevention ($P \geq 0.09$) (Table 2).

In contrast, transformations by low levels of five of the six activated carcinogens were not prevented by retinoid (Fig. 1, protocol IIIb). All tested concentrations of activated carcinogens, benzo[*a*]pyrene-*trans*-7,8-dihydrodiol (BP-diol), BP-diol epoxide, *N*-hydroxy-*N*-2-fluorenylacetylamine (*N*-OH-FAA), *N*-AcO-FAA and 1-methyl-1-nitrosourea (MNU), significantly transformed the cultured glands, except BP-diol epoxide at 10^{-9} M (Table 2). The presence of retinylidene dimedone ($\leq 10^{-5}$ M) during days 4–10 did not significantly inhibit these transformations. *N*-(4-hydroxyphenyl)-all-*trans*-retinamide (HO-phe-ret) at 10^{-6} M and retinyl acetate (10^{-7} M) were also ineffective against transformation by MNU

Table 1 Lack of prevention by early additions of retinylidene dimedone in chemical transformations of cultured mammary glands of mice

Days of treatment	Treatment			Transformed glands*			
	Dimedone (M)	Carcinogen, M	No. of glands	Toxicity (%)†	No.	Incidence (%)	Prevented (%)
0-3	0	None	49	39	1	2	—
	0	BP	10 ⁻⁶	31	12	39	—
	10 ⁻⁷	BP	10 ⁻⁶	32	11	34	13
	0	BP-diol epoxide	10 ⁻⁷	31	6	19	—
	10 ⁻⁷	BP-diol epoxide	10 ⁻⁷	32	9	28	(0)
	0	FAA	10 ⁻⁶	14	3	21	—
	10 ⁻⁷	FAA	10 ⁻⁶	27	8	30	(0)
	0	N-AcO-FAA	10 ⁻⁶	14	5	36	—
	10 ⁻⁷	N-AcO-FAA	10 ⁻⁶	15	5	33	8
	0	None	46	22	1	2	—
3-4	0	BP	10 ⁻⁶	32	10	31	—
	10 ⁻⁷	BP	10 ⁻⁶	32	10	31	0
	0	BP-diol epoxide	10 ⁻⁷	32	6	19	—
	10 ⁻⁷	BP-diol epoxide	10 ⁻⁷	32	11	34	(0)
	0	FAA	10 ⁻⁶	28	6	21	—
	10 ⁻⁷	FAA	10 ⁻⁶	28	7	25	(0)
	0	N-AcO-FAA	10 ⁻⁶	28	4	14	—
	10 ⁻⁷	N-AcO-FAA	10 ⁻⁶	29	5	17	(0)

Mammary glands were treated with retinoid before (days 0-3), or concurrently (days 3-4) with carcinogen. Glands were in development medium during days 0-10, and in regression medium during days 10-24.

*Transformed mammary glands contained nodule-like alveolar lesions, which are the product of the escape from the hormonal controls of alveolar development in whole organ culture. This change to hormone independence, which is brought about by chemical carcinogens, constitutes the operational definition of the chemical transformation^{4,5}. See text for definition of carcinogen abbreviations.

†Per cent of glands with paucity of alveolar buds⁴, resulting in part from uses of the solvents of carcinogens and of retinoid.

(Table 2). Even the presence of retinylidene dimedone (10⁻⁷ M) throughout the entire period of mammary gland development (days 0-10) failed to prevent transformation by equimolar BP-diol epoxide ($P > 0.1$) (not shown).

The nitrosamide, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), was the only reactive carcinogen whose transformation was blocked by retinylidene dimedone. Without retinoid, MNNG had significant transforming activity ($P < 0.02$; Table 2). Acting anti-promotionally in protocol IIIa (Fig. 1), the retinoid at 10⁻⁷ M significantly inhibited transformation by 10-fold and 100-fold molar excesses of MNNG ($P \leq 0.04$).

Thus, mammary gland transformations caused by three procarcinogens, DMBA, BP and FAA, were prevented anti-

promotionally by retinylidene dimedone (Fig. 1). In contrast, transformations by five of six activated carcinogens were not prevented (BP-diol, BP-diol epoxide, *N*-OH-FAA, *N*-AcO-FAA and MNU). Further, 100-fold molar excesses of the procarcinogens overwhelmed the preventive actions of the retinoid. The ability of retinoid to block mammary gland transformations after, but not during or before, the period of treatment with the procarcinogens argues against a mechanism of chemoprevention involving anti-initiation, that is, inhibition of activation of carcinogens or interference at the level of activated carcinogens *per se*. The present findings suggest that the retinoid prevented only those transformations that resulted from very low concentrations of activated carcinogens that were generated endogenously. It is difficult to test this

Fig. 1 Protocols of the transformations of mouse mammary glands by chemical carcinogens, and of the prevention of transformations by retinylidene dimedone (retinoid). The serum-free culture system has been described elsewhere^{4,5,10}. Female BALB/c mice, 3-4 weeks old, were primed with injections of β -oestradiol (1 μ g) and progesterone (1 mg) for 9 consecutive days. On the day after the last injection, mice were killed and their entire second thoracic mammary glands excised and floated on Dacron rafts. The serum-free development medium consisted of Waymouth MB752/1 medium (1 ml per gland) supplemented with 350 μ g ml⁻¹ L-glutamine, 35 μ g ml⁻¹ penicillin G, and 5 μ g ml⁻¹ of each of insulin (I), prolactin (P), aldosterone (A) and hydrocortisone (H). On day 3, the medium was replaced with development medium containing 0.1% (v/v, final) dimethylsulphoxide with or without a listed carcinogen. The medium was renewed without carcinogen 24 h later, and every 2-3 days thereafter as required. Retinylidene dimedone (retinoid) in up to 0.5% ethanol (v/v, final) was present in the development medium during the intervals shown. Control cultures contained matching amounts of dimethyl sulphoxide and/or ethanol. All glands were cultured in the development medium during days 0-10, resulting in full morphological lobuloalveolar development^{2,4}, and then in regression medium containing I (5 μ g ml⁻¹) as the only added hormone for an additional 14 days (days 10-24). Glands were fixed, stained, coded and evaluated^{4,5}. In untransformed glands, all the lobuloalveoli underwent involution (regression). Transformed glands contained one or more nodule-like alveolar lesions that had not regressed. Statistical significance was determined by Fisher's 2 $\times$ 2 exact test.

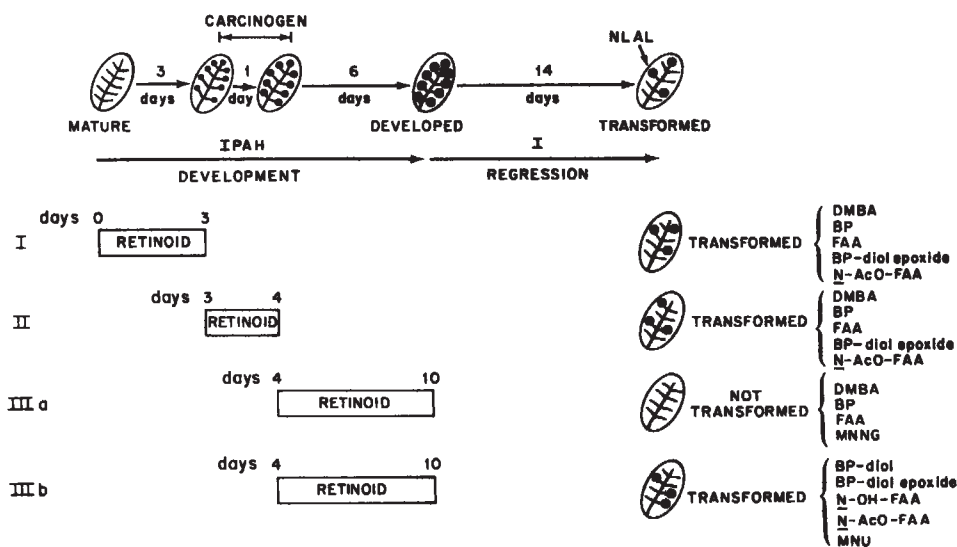


Table 2 Effects of late additions of retinoids on chemical transformations of cultured mammary glands of mice

Carcinogen	Treatment				No. of glands	Toxicity (%)*	Transformed glands		
	M	Dimedone (M)	HO-phe ret (M)	Retinyl acetate (M)			No.	Incidence (%)	Prevented (%)
None	0	0			234	21	1	0.4	—
	0	10 ⁻⁵			58	31	0	0	—
	0	10 ⁻⁷			131	15	1	0.8	—
	0	0	10 ⁻⁶		48	40	0	0	—
	0	0	0	10 ⁻⁷	32	31	0	0	—
BP	10 ⁻⁵	0			29	34	7	24	—
	10 ⁻⁵	10 ⁻⁷			30	30	5	17	29
	10 ⁻⁶	0			32	22	11	34	—
	10 ⁻⁶	10 ⁻⁷			32	19	2	6	82†
	10 ⁻⁷	0			26	23	5	19	—
BP-diol	10 ⁻⁷	10 ⁻⁷			23	9	0	0	100†
	10 ⁻⁷	0			77	38	23	30	—
	10 ⁻⁷	10 ⁻⁵			32	34	8	25	17
	10 ⁻⁷	10 ⁻⁷			35	23	6	17	43
	10 ⁻⁸	0			24	29	6	25	—
BP-diol epoxide	10 ⁻⁸	10 ⁻⁷			26	23	5	19	24†
	10 ⁻⁹	0			26	15	4	15	—
	10 ⁻⁹	10 ⁻⁷			26	23	2	8	47
	10 ⁻⁷	0			56	50	18	32	—
	10 ⁻⁷	10 ⁻⁵			20	40	7	35	(0)
FAA	10 ⁻⁷	10 ⁻⁷			36	44	12	33	(0)
	10 ⁻⁸	0			27	26	3	11	—
	10 ⁻⁸	10 ⁻⁷			26	19	2	8	27
	10 ⁻⁹	0			23	22	2	9	—
	10 ⁻⁹	10 ⁻⁷			23	26	3	13	(0)
N-OH-FAA	10 ⁻⁵	0			18	67	10	56	—
	10 ⁻⁵	10 ⁻⁷			18	28	5	28	50
	10 ⁻⁶	0			25	40	14	56	—
	10 ⁻⁶	10 ⁻⁷			23	22	5	22	61†
	10 ⁻⁶	0			56	39	18	32	—
N-AcO-FAA	10 ⁻⁶	10 ⁻⁵			28	46	6	21	34
	10 ⁻⁶	10 ⁻⁷			28	39	5	18	44
	10 ⁻⁶	0			57	33	18	32	—
	10 ⁻⁶	10 ⁻⁵			27	37	7	26	19
	10 ⁻⁶	10 ⁻⁷			30	30	7	23	28
MNU	10 ⁻⁶	0	0		72	49	15	21	—
	10 ⁻⁶	10 ⁻⁷	0		42	26	9	21	0
	10 ⁻⁶	0	10 ⁻⁶		28	57	10	36	(0)
	10 ⁻⁷	0	0		74	45	13	18	—
	10 ⁻⁷	10 ⁻⁷	0		46	37	10	22	(0)
MNNG	10 ⁻⁷	0	10 ⁻⁶		27	44	5	19	(0)
	10 ⁻⁶	0	0	0	32	56	13	41	—
	10 ⁻⁶	0	0	10 ⁻⁷	31	55	10	32	22
	10 ⁻⁷	0	0	0	32	50	14	44	—
	10 ⁻⁷	0	0	10 ⁻⁷	31	65	11	35	20
MNNG	10 ⁻⁵	0			42	48	16	38	—
	10 ⁻⁵	10 ⁻⁷			42	31	6	14	63†
	10 ⁻⁶	0			40	40	14	35	—
	10 ⁻⁶	10 ⁻⁷			44	34	7	16	54†

Glands were treated with carcinogens during days 3–4, and with retinoid during days 4–10. Mammary glands were in development medium during days 0–10, and in regression medium during days 10–24. The definition of transformed mammary glands is given in Table 1. See text for definition of carcinogen abbreviations.

Per cent of glands with paucity of alveolar buds, resulting in part from uses of solvents of carcinogen and of retinoid.

†Significant prevention according to Fisher's 2 × 2 exact test.

suggestion directly as addition of very low concentrations of activated carcinogens to cultured glands would not result in significant transforming activity due to their consumption in nonspecific interactions with nucleophiles in the medium and mammary gland. Similarly, such low concentration of MNNG presumably survived to cause transformation that was prevented by the retinylidene dimedone, whereas the remainder of the carcinogen was consumed in the nonspecific interactions. The same effect may explain why the feeding of retinyl acetate and HO-phe-ret to rats significantly delayed the appearance of mammary tumours arising from intravenous injections of MNU^{12–14}, whereas these retinoids did not prevent transformation by MNU in the present *in vitro* study.

Two hypotheses may explain the inability of retinoid to prevent mammary gland transformations by most of the activated carcinogens and by high concentrations of procarcinogens. First, the relatively high levels of activated carcinogens from either source may interact with and render

inoperative sensitive elements—such as genes—in the retinoid-action system itself. Second, retinoid may inhibit the early stages of transformation arising from low concentrations of procarcinogens, but fail to block subsequent stages that may result from multiple hits on cellular targets by relatively high concentrations of activated carcinogens.

Retinoids are under intensive study as possible agents for preventing cancer in man^{15–16}. The inability of retinoids to prevent epithelial transformation by activated or direct-acting carcinogens and by high levels of procarcinogens in this system may reflect important limitations of certain anti-promoters in chemoprevention of chemical oncogenesis.

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Histone exchange in chromatin of hydroxyurea-blocked Ehrlich ascites tumour cells

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It is well established that DNA and histone synthesis are tightly coupled^{1,2}. Nevertheless, these two processes can be partially uncoupled by drugs specifically inhibiting protein^{3,4} or DNA^{5,6} synthesis, and also during *n*-butyrate-induced differentiation of Friend cells⁷. The fate of the histones synthesized in the absence of DNA synthesis is unknown; they could be: (1) degraded without joining chromatin; (2) deposited on chromatin as extra histones; or (3) replace original chromatin histones. The only data concerning this problem are a recent report⁸ supporting the second possibility. We present evidence here in favour of the third possibility by showing that the histones synthesized in the absence of DNA synthesis enter chromatin and become organized in nucleosomes.

Exponentially growing Ehrlich ascites tumour (EAT) cells were incubated *in vitro* with 5 mM hydroxyurea (HU). At different intervals, samples from control and HU-treated cells were taken, incubated for 30 min with ³H-thymidine and ¹⁴C-protein hydrolysate and the specific radioactivities of the histones and DNA in the isolated chromatin⁸ were determined. In full agreement with the reported effect of HU on other cell lines⁵, DNA synthesis was almost completely inhibited within an hour of HU treatment and remained low for several further hours (Fig. 1). At the same time the incorporation of ¹⁴C-labelled amino acids continued in all five histones of chromatin, although at a reduced rate amounting to 30-40% of the corresponding controls (Fig. 1). This result shows that the histones synthesized during the HU block (HU-histones) have joined chromatin. It was reported that these histones were deposited as extra histones onto certain chromatin fractions which could be separated from the rest of chromatin on the basis of their higher protein-to-DNA ratio⁵. To check this possibility chromatin from control and HU-treated cells was extensively fragmented by sonication and run on shallow metrizamide density gradients. Both chromatins showed certain heterogeneity with respect to the protein-to-DNA ratio of the fragments (Fig. 2), reflecting the presence in total chromatin of fractions with different non-histone protein

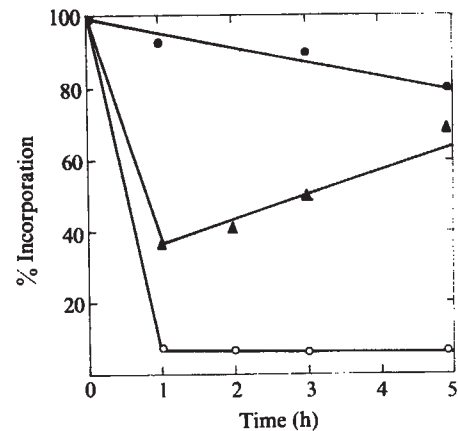


Fig. 1 Effect of HU on the synthesis of DNA and histones in EAT cells. On day 7 after intraperitoneal inoculation of EAT cells to Agnes-Blum white mice, the ascites liquid was obtained in sterile conditions and 5-ml aliquots were mixed with 50 ml of Gibco carbonate-free 199 medium containing 50 mM HEPES buffer (BDH), pH 7.1. The mixture was incubated at 37 °C under gentle shaking. Hydroxyurea (Serva) was added to a final concentration of 5 mM and at the specified time intervals 5-ml aliquots were taken and incubated with 2 μ Ci ³H-thymidine (11 Ci per mg) and 10 μ Ci of ¹⁴C-protein hydrolysate (1.5 mCi per mg) per ml for 30 min. The samples were cooled on ice and immediately used for isolation of chromatin by extraction of Nonidet P40 nuclei with increasing salt concentrations up to 0.35 M NaCl (ref. 8). The final pellet was dissolved in deionized water and DNA determined spectrophotometrically assuming 1 A₂₆₀ = 50 μ g of DNA. For radioactivity measurement 0.5-ml samples were mixed with 5 ml of a toluene-Triton X-100 (2:1) scintillation mixture²⁰ and differentially counted for ³H and ¹⁴C in a LKB Ultrabeta 1210 liquid scintillation counter. Histones were extracted from chromatin with 0.5 M H₂SO₄ in the cold and precipitated with ethanol. They were resolved by electrophoresis in 15% polyacrylamide gels containing 0.9 M acetic acid-2.5 M urea¹⁰. The gels were stained with Amido black, scanned at 580 nm with a gel scanner (ISCO 1310) and cut into 2-mm slices. The slices were dissolved in 0.5 ml of 25% NH₄OH-30% H₂O₂ (1:4) mixture at 60 °C and counted as above. The histone fractions were pooled together and the specific radioactivity of the total histone was determined in arbitrary units as the ratio between the total counts and the area under the peaks. ●, Specific radioactivity of DNA from control cells; ○, specific radioactivity of DNA from HU-treated cells; ▲, specific radioactivity of total histone from HU-treated cells.

content. However, the sedimentation profile of the HU-treated chromatin was identical to that of control chromatin and showed no additional fraction comprising predominantly the labelled proteins. This disputes the theory that HU-histones are deposited as extra histones.

The fate of the HU-histones was further studied using micrococcal nuclease. Isolated nuclei⁹ from HU-treated cells labelled for 90 min with ¹⁴C-protein hydrolysate were digested with micrococcal nuclease to give about 15% acid-soluble DNA and the digest was run on linear 5-30% sucrose density gradient. Histones were isolated from different fractions of the gradient and from the undigested pellet and their specific radioactivities were determined. They were the same in all fractions and in the pellet, which suggested that the HU-histones were organized in nucleosomes indistinguishable from the normal chromatin nucleosomes.

To confirm this point we studied isolated nucleosomes from HU-treated and control chromatin. They had the same total protein-to-DNA ratio of 1.15:1.00, histone-to-DNA ratio of 1:1, and a full complement of nucleosomal histones as judged by acetic acid-urea polyacrylamide gel electrophoresis¹⁰ (not shown). These nucleosomes were analysed by sucrose density gradient and by equilibrium metrizamide density gradient centrifugation. In both gradients HU-treated nucleosomes co-sedimented with the control nucleosomes (Fig. 3).

The metrizamide density gradient is more sensitive to changes in the protein-to-DNA ratio. It has been shown (and confirmed in our experiments) that there is a linear relationship between this ratio and the buoyant density of the deoxyribonucleoprotein¹¹. This relationship shows that the addition of one histone per nucleosome (which would increase the protein-to-DNA ratio of our particles by 10%) will increase their buoyant density by 0.01 g cm^{-3} . To see how this increase would affect the ^3H - and ^{14}C -labelled peaks in the density gradient, the following points should be taken into consideration: (1) The ^3H peak represents a small fraction of DNA pulse-labelled before the HU block; (2) the ^{14}C peak also corresponds to a small fraction of histones synthesized after the HU block; and (3) if this histone fraction is additionally adsorbed it would be distributed at random among nucleosomes containing labelled and nonlabelled DNA. Thus it can be deduced that if the ^{14}C -histones bind to the nucleosomes as extra protein the whole ^{14}C -labelled peak in Fig. 3d would be shifted to the left, while the ^3H -labelled peak would remain practically unaffected due to the small percentage of (^{14}C , ^3H)-labelled heavy particles.

This shows that in our conditions of labelling the addition of new histone molecules to the nucleosomes would cause a shift of the ^{14}C peak relative to the ^3H peak. Such a relative

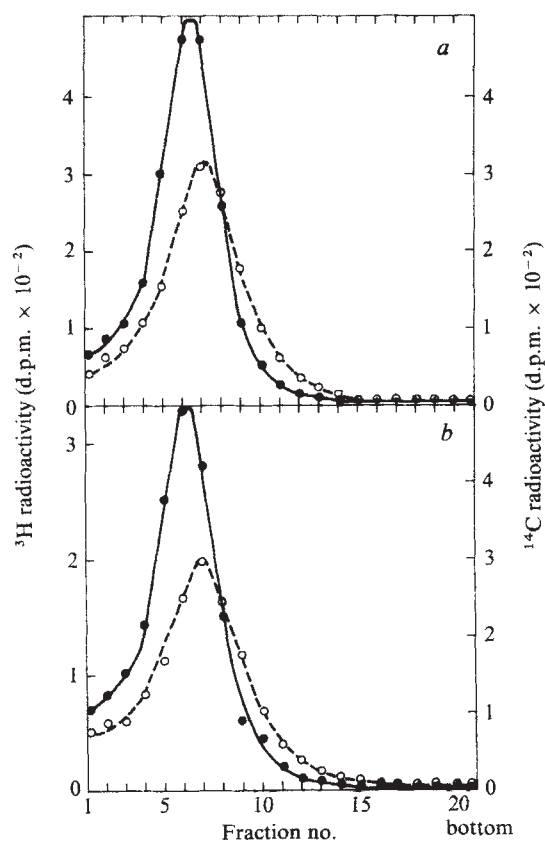


Fig. 2 Equilibrium metrizamide density gradient centrifugation of control (a) and HU-treated (b) EAT chromatin. EAT cells were grown *in vitro* in the presence of 5 mM HU for 5 h and then labelled with ^3H -thymidine and ^{14}C -protein hydrolysate as described for Fig. 1. Control and HU-chromatins were fragmented by sonication with a MSE ultrasonic unit at setting 1A for 5 min ($5 \times 1 \text{ min}$ in an ice bath) to give fragments containing 200–1,000 base pairs of DNA. Aliquots (0.5-ml) were mixed with 4 ml of 45% metrizamide (Nyegaard) in 1 mM EDTA, 5 mM Tris-HCl, pH 8 to a final concentration of 40% metrizamide. The tubes were filled up with liquid paraffin and spun in the Beckman Ti 50 rotor at 33,000 r.p.m. at 4°C for 65 h. The gradients were unloaded with a LKB peristaltic pump from the bottom and after 1:4 dilution with distilled water were counted for ^3H and ^{14}C as described for Fig. 1. ●—●, ^3H -DNA; ○—○, ^{14}C -protein.

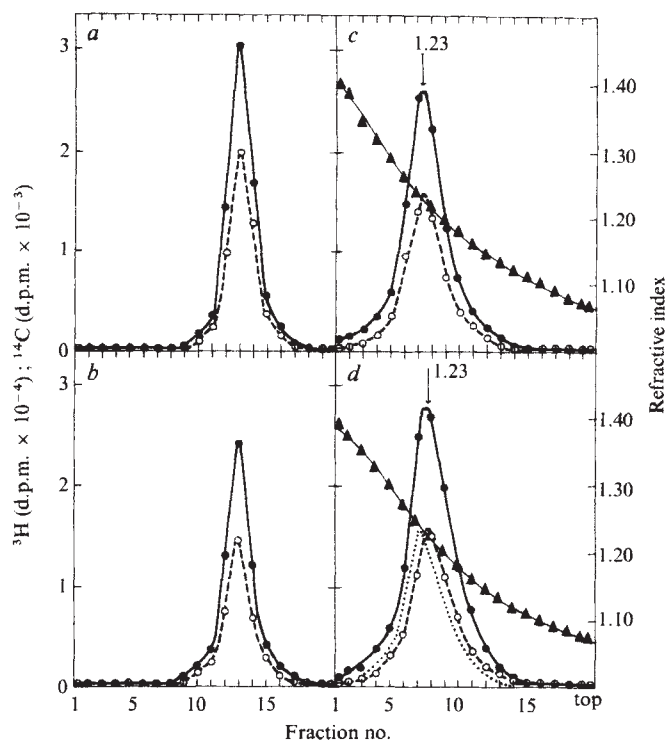


Fig. 3 a, b, Sucrose density gradient, c, d, equilibrium metrizamide density gradient centrifugation of nucleosomes from control (a, c) and HU-treated (b, d) chromatin. EAT cells were incubated with ^3H -thymidine for 30 min to prelabel DNA, treated with HU for 1 h and labelled with ^{14}C -protein hydrolysate for 90 min as described for Fig. 1. Control cells were labelled according to the same protocol, omitting the HU treatment. Nuclei were isolated by treating the cells with Nonidet P40 at low ionic strength⁹ in the presence of 1 mM phenylmethylsulphonyl fluoride (PMSF) (Serva). They were suspended in 1 mM CaCl_2 , 10 mM Tris-HCl, pH 8 to make $40\text{--}50 \text{ A}_{260} \text{ ml}^{-1}$ and were digested with 2 EU of micrococcal nuclease (Boehringer) per A_{260} unit at 37°C for 10 min. The reaction was stopped by adding EDTA to a final concentration of 2 mM EDTA, pH 8. Samples (4 ml) were layered on 5–30% linear sucrose density gradients containing 1 mM EDTA, 5 mM Tris-HCl, pH 8. The gradients were centrifuged in the Beckman SW 27 rotor at 26,000 r.p.m. at 4°C for 18 h and unloaded from the bottom with a LKB peristaltic pump. Aliquots from each fraction were diluted and differentially counted as described in Fig. 1 legend. The fractions containing the mononucleosome peak were pooled together and precipitated with 5 mM CaCl_2 (ref. 21). After 2 h in the cold, the precipitated nucleosomes, which represented about 80–90% of the total A_{260} of the fractions, were collected by centrifugation and resuspended in 2 mM EDTA, pH 8 containing 1 mM PMSF. Samples (0.5 ml) were layered on 5–20% linear sucrose density gradients prepared as above and were run in the Beckman SW40 rotor at 38,000 r.p.m. at 4°C for 20 h. The homogeneous material from the middle of the sucrose gradients was used for the equilibrium centrifugation which was performed exactly as described in Fig. 2 legend, with the only difference that the final concentration of metrizamide was 35%. The gradients were fractionated and counted as described for Figs 1 and 2 and the buoyant densities of the metrizamide gradient fractions were calculated from the corresponding refractive indices²². ●—●, ^3H -DNA; ○—○, ^{14}C -protein;, calculated position of the ^{14}C -peak if the protein-to-DNA ratio increased by 10% (one extra histone per nucleosome).

shift would be easily detected because ^3H and ^{14}C counts are measured in the same double-labelled fraction of the density gradient and the $^{14}\text{C}/^3\text{H}$ ratio would change significantly. If a nucleosome binds one extra histone per molecule the shift of the ^{14}C peak relative to the ^3H peak could be detected as shown in Fig. 3d and the $^{14}\text{C}/^3\text{H}$ ratio of the fractions will change significantly (a decrease of 20–40% in the right shoulder of the peak).

Note that these changes in the position of the ^{14}C peak and $^{14}\text{C}/^3\text{H}$ ratio should be regarded as minimal estimates, for adsorption of single histone molecules on the nucleosomes would hardly occur *in vivo*. It has been shown that histones can form dimers¹², tetramers¹³ and octamers^{14,15} and that they join chromatin as such preformed stoichiometric complexes. This is demonstrated by the fact that during chromatin replication, old and new histones do not mix¹⁶ and that both *in vitro*^{17,18} and *in vivo*¹⁹, H3/H4 tetramers and H2A/H2B dimers sequentially enter chromatin. All these data strongly indicate that it is unlikely that individual newly synthesized histone molecules would be found in the nucleus and if new histones in HU-chromatin join nucleosomes as additional protein they would be adsorbed as dimers and tetramers which will shift the ^{14}C peak in Fig. 3d still further to the left. However, as can be seen, this peak coincides exactly with the peak of ^3H -DNA, and the $^{14}\text{C}/^3\text{H}$ ratio of all fractions is the same as in the corresponding control nucleosomes. This eliminates the hypothetical situation of even one extra histone per nucleosome.

Our results show that the HU-histones are not additionally bound to already existing nucleosomes, but are present in chromatin as components of octamers associated with the old (prelabelled) DNA. This result can be explained by assuming that the newly synthesized histones in HU-arrested cells have replaced some of the old histones, which in the conditions of

HU-block have dissociated from DNA. It contradicts the current concept that, once incorporated in chromatin, histones cannot be replaced, which seems to be true for normal situations only.

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Spectrin tetramer-dimer equilibrium and the stability of erythrocyte membrane skeletons

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The inner side of the red-cell membrane is laminated by a two-dimensional network of membrane proteins which include spectrin, actin and some other components¹⁻⁴. After extraction of lipids and integral proteins from the membrane, this membrane skeleton can be visualized as a ball-shaped network consisting of twisted fibres¹⁻⁴ and globular protrusions⁴; however, the assembly of the individual proteins in the membrane skeleton is not well understood. Spectrin can be eluted from the membrane in the form of dimers and tetramers⁵⁻⁸. Electron microscopic study with low-angle shadowing technique shows that spectrin dimers are two parallel strands of twisted fibres presumably representing bands 1 and 2 of spectrin⁹. Spectrin tetramers presumably formed by head-to-head associations of two dimers are twice as long⁹. In solution, the spectrin dimer-tetramer equilibrium depends on temperature and salt concentration^{7,8}; however, it is not known whether the same equilibrium exists in the membrane and whether it affects the physical properties of the membrane, such as its structural stability and deformability. We now demonstrate that spectrin dimers and tetramers are in a reversible equilibrium in the membrane and that in physiological conditions this equilibrium favours spectrin tetramers. Furthermore, we show that transformation of spectrin tetramers to dimers, as induced by ghost incubation in hypotonic conditions, diminishes the structural stability of the Triton-insoluble membrane skeletons.

We have altered the equilibrium between spectrin tetramers and dimers in the membrane by exposing ghosts to changes in temperature and ionic strength which alter spectrin dimer-tetramer equilibrium in solution. After stabilization of the equilibrium by cooling of ghosts to 0°C, spectrin was extracted from the red-cell ghosts by overnight dialysis in the

cold against a low ionic strength buffer. The amount of spectrin dimers and tetramers was measured by gel filtration on a 4% agarose column⁵. The spectrin tetramers and dimers could also be resolved by electrophoresis at 4°C on agarose/acrylamide composite gels in a buffer containing 40 mM Tris-HCl pH 7.4, 20 mM Na acetate, 2 mM mercaptoethanol and 2 mM EDTA.

About 80% of the spectrin was extracted from freshly isolated red-cell ghosts by overnight dialysis in the cold against low ionic strength buffer. The extracted spectrin was mostly in the form of tetramers (Fig. 1, peak II), and a high molecular weight void volume complex (Fig. 1, peak I) as previously observed by others^{7,8}. After a subsequent incubation of this extract at 37°C for 15 min in isotonic conditions, about 60% of the void volume complex and 70% of spectrin tetramers were dissociated and recovered mostly as spectrin dimers (Fig. 1, peak III). In contrast, incubation of intact ghosts rather than spectrin extracts in identical conditions (37°C, 15 min) in isotonic buffer failed to transform spectrin tetramers to dimers as indicated by the elution profile of crude spectrin extracted from such ghosts (Fig. 2a).

In contrast to a relative stability of spectrin tetramers in ghosts incubated in isotonic conditions, ghost incubation at 37°C for 15 min at low salt concentrations (5 mM sodium phosphate pH 7.4, 10-30 mM NaCl), resulted in a dissociation of spectrin tetramers to dimers; this transformation was stimulated by a decrease in salt concentration (Fig. 2b-e). At 37°C, an equilibrium between the two species was reached in minutes, as is observed in solution^{7,8}.

The supernatants of ghosts incubated either at isotonic or hypotonic conditions (37°C, 15 min) contained only very small amounts of spectrin (1-3%) indicating that during such short incubation spectrin was not released from the membrane. Furthermore, the total amount of spectrin extracted from the membrane (80%) was the same in fresh ghosts containing mostly spectrin tetramers and in hypotonically pretreated ghosts (5 mM Na phosphate pH 7.4, 10 mM NaCl, 1 mM mercaptoethanol, 37°C, 15 min) in which spectrin tetramers were converted to dimers. After preincubation in isotonic conditions spectrin extractability decreased from 80% to about 65%. However, this 15% difference in spectrin extractability cannot account for the fact that in hypotonic conditions over 40% of spectrin was present in a dimer form. Thus, the

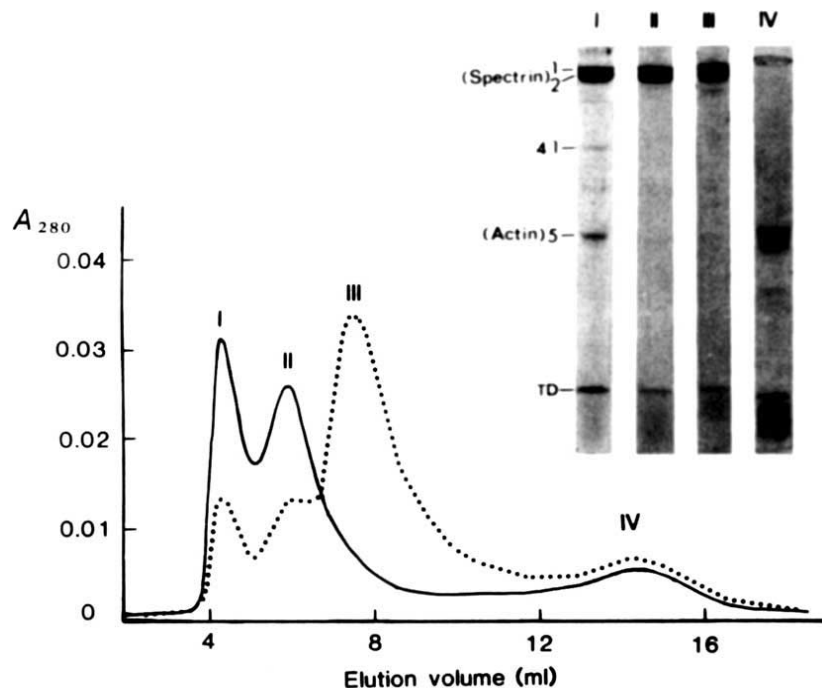


Fig. 1 Demonstration of spectrin transformation in solution by gel filtration analysis of crude spectrin incubated at 37°C in isotonic conditions. Crude spectrin from fresh human erythrocytes was prepared by dialysis of ghosts³¹ against 0.1 mM Na phosphate, 0.1 mM EDTA pH 8.0, at 0–4°C for 20–30 h. The fragmented ghosts were pelleted by centrifugation at 250,000g for 30 min at 4°C. Crude spectrin supernatants (0.7 ml, $A_{280}=0.6$) with (---) and without (—) further incubation in isotonic conditions (5 mM Na phosphate, 150 mM NaCl, 5 mM mercaptoethanol, 5 mM EDTA pH 7.5, 37°C, 15 min) were eluted in the cold from the Bio-Gel A-15 m (200–400 mesh) agarose column (1 cm × 40 cm) with 10 mM Na phosphate, 150 mM NaCl, 5 mM mercaptoethanol, 5 mM EDTA pH 7.5 (ref. 5). Protein in the effluent was monitored by absorbance at 280 nm. Gel electrophoresis of proteins in fraction I (the void volume fraction containing polymerized complexes of spectrin, actin, and band 4.1), II (spectrin tetramers^{5,7}), III (spectrin dimers^{5,7}), and IV (mostly actin) was carried out in the system of Laemmli³².

changes in the amount of spectrin tetramers and dimers in ghosts pretreated in various conditions are likely to reflect alterations in the equilibrium between the two spectrin species in the membrane *in situ* rather than a selective extraction of one of the two spectrin species from the membrane.

The transformation is readily reversible: spectrin dimers reassociate into tetramers in the membrane after restoration of isotonicity at 37°C (Fig. 2f). Such reversibility indicates that spectrin tetramers are the physiological species and that the transformation of spectrin tetramers to dimers in the membrane in hypotonic conditions is a consequence of thermodynamic equilibrium but not a result of proteolytic degradation.

It is not surprising that at physiological ionic strength and temperature, spectrin tetramers are the predominant species in the membrane (Fig. 2a), whereas spectrin dimers predominate in identical conditions in solution (Fig. 1). Spectrin at the membrane surface is highly concentrated [probably about 230 mg ml⁻¹ (ref. 10), that is about 100 to 1,000 times more than the concentration typically used in the *in vitro* studies of spectrin dimer-tetramer equilibrium]. Furthermore, membrane-associated spectrin is confined to two dimensions thus having a lower entropy than spectrin in solution.

The absence of higher self-association states of spectrin (such as hexamers or octamers) in the membrane and the presence of high molecular weight complexes ($mw > 1.5 \times 10^7$) containing spectrin, actin and band 4.1 (Fig. 1) further suggest that spectrin tetramers are cross-linked non-covalently by other skeletal components such as actin and band 4.1 in the membrane, as recently also suggested by others^{11–15}.

To test the role of spectrin tetramer-dimer equilibrium in maintaining membrane structural integrity, we varied the ratio of spectrin dimers to tetramers in ghosts by incubating them at 37°C in hypotonic conditions and subsequently examined the susceptibility of such ghosts or their Triton-insoluble membrane skeletons to fragmentation by mechanical shaking. Ghosts exposed to hypotonic conditions at 37°C were not fragmented after a vigorous shaking (40 min, 1,550 oscillations per min). However, membrane skeletons prepared from such ghosts were more easily fragmented by shaking (Fig. 3f) than the skeletons from untreated ghosts (Fig. 3c). The instability of membrane skeletons exhibited a similar dependence on salt concentration in the medium as did the spectrin dimer-tetramer equilibrium in the native membranes. The decrease of

skeleton stability was noted in Triton-extracted samples prepared at both 25°C and 0°C.

Membrane skeletons and skeleton fragments in suspension were best visualized after staining with uranyl acetate.

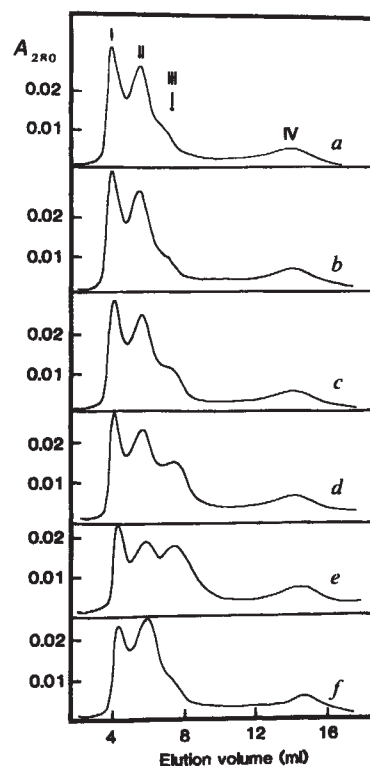
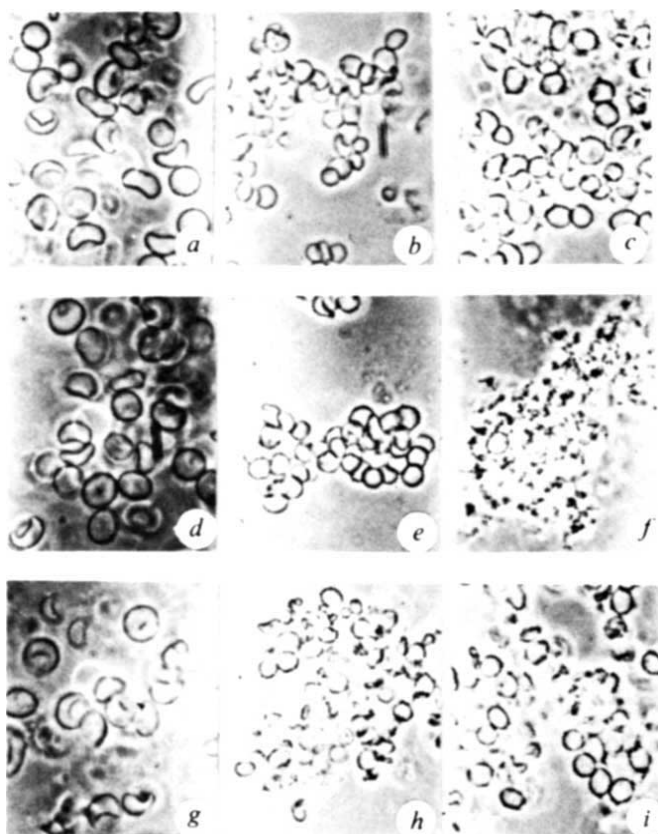


Fig. 2 Demonstration of spectrin transformation in the erythrocyte membrane by gel filtration analysis of crude spectrin extracts from ghosts incubated in hypotonic conditions. Before low ionic strength extraction and fractionation in the cold, as described in Fig. 1, ghost cells were incubated at 37°C for 15 min in a buffer containing NaCl at 150 mM (a), 40 mM (b), 30 mM (c), 20 mM (d) and 10 mM (e). f Is the elution profile of crude spectrin derived from isotonic-buffer-reincubated ghosts (5 mM Na phosphate, 150 mM NaCl, 1 mM mercaptoethanol 37°C, 15 min) which were previously hypotonic-buffer-incubated as in e.



RBC ghosts Membrane skeletons Mechanically shaken membrane skeletons

Fig. 3 Diminished stability of membrane skeletons from ghosts incubated at 37°C in hypotonic conditions. *a*, Untreated; *d*, hypotonic-buffer-incubated (as in Fig. 2*e*); and *g*, isotonic-buffer-reincubated (as in Fig. 2*f*) ghosts were incubated at 25°C for 15 min with two volumes of Triton X-100 solution¹ (3% Triton X-100 in 5 mM Na phosphate, 1 mM mercaptoethanol pH 7.4) to remove most lipids and integral proteins from the ghosts. Aliquots (200 µl) of skeleton suspensions from these three samples were transferred to small test tubes (0.7 cm × 7 cm), mounted horizontally on a pipette shaker (Clay Adams, 1,550 oscillations per min) and examined morphologically before (*b*, *e*, and *h*, respectively), and after (*c*, *f*, and *i*, respectively) shaking (linear travel of 4 mm per oscillation) for 40 min at 4–6°C. The membrane skeleton suspension (5 µl) was taken out of the tube, applied onto a siliconized glass slide, gently mixed with 1% uranyl acetate (5 µl), covered with a siliconized cover slip, and examined under the phase contrast light microscope. A gross disintegration (>90%) of membrane skeletons derived from ghosts incubated in hypotonic conditions was usually noted after shaking for about 30–50 min (40 min in Fig. 3*f*), whereas those derived from untreated or isotonic-buffer-reincubated ghosts usually remained intact (>90%).

Although uranyl acetate artefactually induces a clumping and shrinking of membrane skeletons (Fig. 3*b*, *e*), the disintegration of skeletons into fragments can be easily recognized (Fig. 3*f*). A low concentration of uranyl acetate was used to avoid the precipitation of integral proteins from the suspension. Similar changes in stability of membrane skeletons were also observed in unstained skeletons after addition of >30 mM NaCl. NaCl did not induce skeleton clumping but it produced much less contrast for examination by phase contrast light microscopy.

Reincubation of the spectrin dimer enriched ghosts in isotonic conditions, which resulted in a reassociation of spectrin dimers to tetramers in the membrane (Fig. 2*f*), restored the skeletal stability to normal (Fig. 3*i*). Since no more than 1–3% of spectrin is released into the supernatant in such conditions, we conclude that the transformation of

spectrin itself rather than the reattachment of spectrin to the membrane was responsible for the restoration of membrane skeleton stability.

Previous work suggests that the stability of membrane skeletons depends on the state of spectrin phosphorylation¹⁶. We have therefore explored the effect of red cell metabolic depletion on the spectrin tetramer dimer equilibrium in the membrane. We have incubated intact red cells under nitrogen at 37°C without metabolic substrates for 16 h to deplete red-cell ATP levels to less than 10% of preincubation values. ATP depletion in such cells did not alter the proportion of spectrin tetramers and dimers in freshly prepared ghosts or in ghosts subsequently subjected to incubation at 37°C in isotonic or hypotonic conditions (data not shown). Thus, the spectrin tetramer–dimer equilibrium is probably not influenced by spectrin phosphorylation–dephosphorylation⁸.

A decrease in membrane skeleton stability has recently been reported after treatment of skeletons with DNase-I¹⁵, which is associated with a depolymerization of actin oligomers in the membrane skeleton. Thus, it seems that both spectrin tetramers and actin oligomers may serve as important structural links in the membrane skeleton. This is consistent with the recent membrane skeleton model^{10,17,18}, which postulates flexible spectrin tetramer fibres connected into a two-dimensional network by oligomeric complexes of actin and band 4.1. Recent evidence further indicates that this skeletal network is linked to the erythrocyte membrane through interaction of spectrin with ankyrin or syndein^{19–22} (band 2.1, which anchors spectrin to the major transmembrane protein, band 3), interaction of spectrin with negatively charged phospholipids²³, and binding of oligomeric actin to unidentified membrane binding sites²⁴. Cross-linking studies also suggest a proximity of band 1 of spectrin and band 3 in the membrane²⁵. It is possible that these interactions may strengthen skeleton stability *in situ* and hence, account for a normal mechanical stability of spectrin dimer enriched ghosts. It remains to be determined whether the altered spectrin dimer–tetramer equilibrium further affects other membrane phenomena such as the distribution and mobility of intramembrane particles^{26–29} and glycoproteins³⁰, or membrane deformability.

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Is resistance of a muscle to fatigue controlled by its motoneurones?

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The original experiment of Buller *et al.*¹ and the many subsequent confirmatory reports clearly show that the time-to-peak tension and many other speed-related parameters of slow and fast muscle fibres are dictated by the motoneurone². It has been concluded that the motoneurone exerts this control of the physiological and associated biochemical properties by the frequency at which it excites the muscle fibre³. However, no studies have been reported on the fatigue properties and the associated biochemical characteristics after cross-reinnervation. Based on the 'size principle' of motoneurones⁴, it would be reasonable to assume that a muscle fibre reinnervated by a small motoneurone would be active often and that this would be manifested biochemically as an elevated oxidative capacity⁵. Also, it has been shown repeatedly that the mitochondrial content of a muscle fibre can be modified by daily endurance type exercise⁶. Thus, it would seem that the motoneurone at least indirectly also controls the mitochondrial content of a muscle fibre by controlling the degree of activity. We have now tested this hypothesis using self- and cross-reinnervated muscles in cats. We found that fast- and slow-twitch muscles retained their characteristic fatigue resistance properties regardless of whether the nerve to which they had become connected had originally innervated a fatigue-resistant or relatively fatiguable muscle.

The soleus and flexor hallucis longus (FHL) muscles of nine cats were denervated and reinnervated with their own nerve or with the nerve which had formerly innervated the other muscle. One leg was always self-reinnervated while the contralateral limb was cross-reinnervated. After 12–14 months, 70 single motor units were isolated through ventral root dissection and studied as described by Goslow *et al.*⁷. Histochemical analyses were carried out as reported by Edgerton *et al.*⁸.

Six of the nine animals produced muscles which had been reinnervated by both the contralateral and original nerves. Such a preparation was seen for six soleus muscles and two FHL muscles. Careful dissection distally from the point of original nerve transection facilitated the placement of stimulating electrodes on each of the two muscle nerves. Thus, confirmation of the isolation of an α axon belonging to either a self- or cross-reinnervated motor unit was provided by a record of an all-or-none action potential from the spinal nerve filament following stimulation of the distal whole muscle nerve.

The fatigue test, carried out at the end of the experiment, consisted of a 330-ms train of impulses delivered every second. The impulse frequency within each train was 40 Hz (ref. 9). These trains were continued for 4 min. The fatigue index chosen was the ratio, expressed as a percentage, of the accumulated peak tension during the first 2 min divided by the accumulated peak tension during the entire 4-min^{10,11} test. This ratio ranges from 100% in a completely fatiguing muscle to 50% in one which is completely resistant to fatigue. Values below 50% indicate potentiation of the unit's response during the 4-min period.

The relationship between the distribution of times to peak tension of the isolated motor units and their fatigue indices is shown in Fig. 1. Based on the proportion of muscle

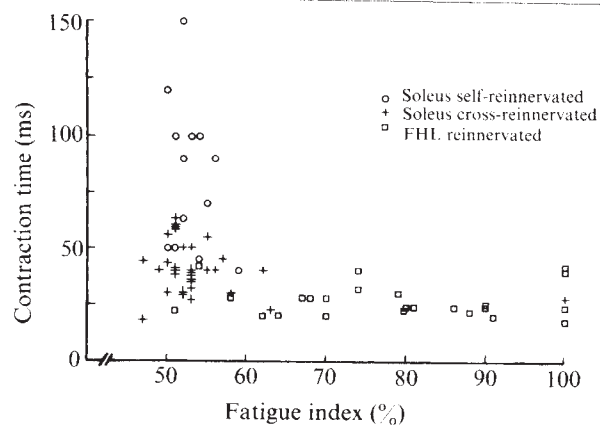


Fig. 1 The relationship between contraction time and the fatigue index (% see text) for the soleus self-reinnervated ($n=13$), soleus cross-reinnervated ($n=33$) and FHL self-reinnervated ($n=24$) motor units. Note that, with the exception of one cross-reinnervated soleus unit, all soleus units, either self-reinnervated or crossed with the nerve of the FHL, exhibit a similar resistance to fatigue.

histochemical fibre types¹² and motor unit types¹³ in the normal FHL muscle, if the motoneurone was responsible for the endurance properties of the innervated fibres, approximately 65–70% of the cross-reinnervated motor units in the soleus would have been expected to have demonstrated considerable fatigue. However, of the 33 soleus cross-reinnervated motor units studied, only one unit became severely fatigued. The profile of fatigue indices for all other cross-reinnervated units (mean of 52%) was almost identical ($P>0.05$) to that for the self-reinnervated soleus units (mean of 53%): all of these units were highly resistant to fatigue. In contrast, the 24 motor units from the self-reinnervated flexor muscles exhibited a wide spectrum (51–100%) of fatigue indices (mean 78%), which tallies well with histochemical¹² and physiological¹³ findings for normal FHL muscle.

The tension and electromyographic potential from the stimulation pulses from a soleus motor unit reinnervated by a foreign flexor nerve are shown in Fig. 2. Note that the first and last trains of impulses induced similar peak forces in the cross-reinnervated soleus motor unit. Additionally, this unit showed no sag property¹⁴ when stimulated at a sub-fusion frequency.

Consistent with the high resistance to fatigue in the cross-reinnervated soleus units, the histochemical population of these units consisted solely of slow-oxidative- and fast-oxidative-type fibres¹⁵, as shown in Fig. 3. Also, for those soleus cross-reinnervated fibres that stained lightly with myofibrillar ATPase, pH 10.4, the staining profile for the glycolytic enzyme α -glycerophosphate dehydrogenase (Fig. 3c)

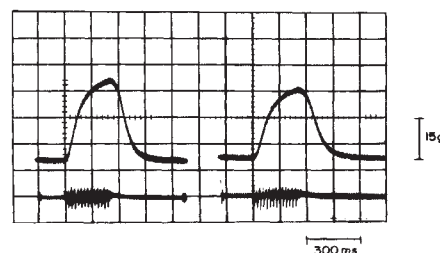


Fig. 2 The fatigue response of a soleus motor unit cross-reinnervated with an FHL nerve. The contraction on the left represents the initial tetanic tension developed (20g) in response to a 40-Hz train of impulses lasting 330ms, and the contraction on the right illustrates the tension developed at the end of the 4-min fatigue test (see text). The fatigue index of this unit was 50%. The time to peak tension was 40ms, and its twitch tension was 1.2g. The twitch was potentiated by 50% with a tetanus; no 'sag' of the tetanus was evident.

was completely transformed to that expected for fast-oxidative-glycolytic fibres¹⁵. This type of enzyme profile is rare in the normal soleus muscle¹². Interestingly, the soleus muscle in which the fatiguable unit was found did not differ histochemically from the other soleus cross-reinnervated muscles. In no instance did a cross-reinnervated fibre that stained darkly for myofibrillar ATPase not stain darkly also for α -glycerophosphate dehydrogenase. The reverse was not always true. The remaining cross-reinnervated fibres maintained the enzyme profile typical of a normal cat soleus muscle. No fibres could be classified as fast twitch glycolytic in self- or cross-reinnervated soleus muscle. Fibres in the self-reinnervated soleus and FHL (Fig. 4) muscles rarely contained any enzyme profile different from those observed in the normally innervated muscles¹².

There are several possible explanations for the maintenance of the fatigue resistance properties of the soleus muscle cross-reinnervated by a nerve that normally innervates a relatively fatiguable muscle. Based on normal data¹², one would expect that approximately 70% of the motor units would be very fatiguable assuming that the reinnervation process was random. The possibility that the motor units that innervate the fatigue-resistant muscle fibres may have reinnervated more effectively is not supported by the normal ratio of motor unit and histochemical types seen after self-reinnervation in the FHL muscle (Fig. 4). A second plausible explanation is that whatever the variable associated with excitation of the muscle (pattern or degree of activity) that one might assume to have changed was not actually modified by the cross-reinnervation. However, if this were the case, the major underlying assumption of the cross-reinnervation study by Salmons and Sreter³ and many others who conclude that impulse pattern was the factor regulating the physiological and contractile properties would require re-evaluation. A third and perhaps

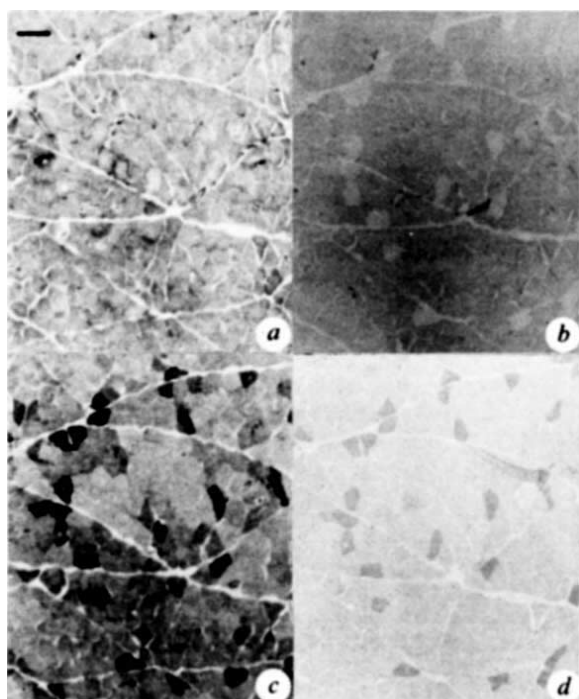


Fig. 3 Serial cross-sections of a cross-reinnervated soleus muscle stained for NADH-D (a), myofibrillar ATPase, pH 4.35 preincubation (b), α -glycerophosphate dehydrogenase (c) and myofibrillar ATPase, pH 10.4 preincubation (d). Note the scattered array of a relatively few fibres staining intensely for ATPase (pH 10.4) and a rather high and homogeneous staining for NADH-D. Note also that all the darkest-staining fibres in c correspond to the dark fibres in d and the light ones in b, whereas moderately stained fibres in c cannot be identified in d. Fibre type grouping was the exception rather than the rule in all self-reinnervated and cross-reinnervated muscles. Scale bar in upper right corner indicates 100 µm.

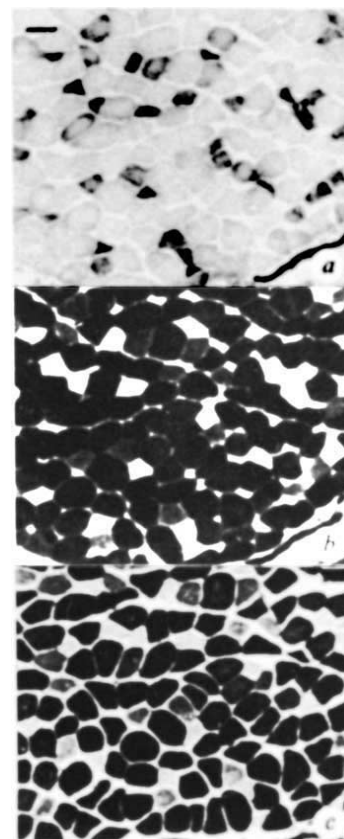


Fig. 4 A high percentage of low NADH-D-staining fibres are seen in the self-reinnervated FHL muscle (a), consistent with the concept that many of the FHL axons that reach the soleus would be expected to result in a high proportion of easily fatigued units. Figure 4b illustrates the ATPase, pH 10.4 preincubation, and c, the ATPase, pH 4.6 preincubation, for the same muscle. These staining profiles are consistent with normal muscle. Scale bar in upper right corner indicates 100 µm.

more plausible interpretation is that the basic genetic programme which regulates mitochondrial content in a muscle fibre is determined to a major extent by factors within the fibre. Although the quantity of mitochondria can be readily modified by stimulation¹⁶ or by introducing an exercise training programme⁶, this does not justify the assumption that activity level is the sole or even basic mechanism dictating mitochondrial concentration in a muscle fibre. This interpretation is supported by the finding that the normal array of fatigue properties and histochemical profiles in soleus and gastrocnemius motor units still exists 3 months after a low thoracic complete transverse section of the spinal cord¹⁷.

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MATTERS ARISING

Bounds on food web connectance

REJMANEK AND STARÝ¹ have shown that the product of the number of species in a community (m) and the proportion of possible interactions (C) is a constant; that is, their relationship is hyperbolic. Though the authors present this as only an empirical result it is consistent with May's² result derived from considerations of model stability. However, an alternative, perhaps simpler explanation suffices. Suppose that each species in a community has a number of predator and prey species independent of the number of species in the community. The number of interactions in the system scales in proportion to the number of species in the system. However, the potential number of interactions scales as $m(m-1)$, if we exclude intraspecific effects. Thus, the connectance scales as $1/(m-1)$ which, for large m will be indistinguishable from the result predicted by May². Such hyperbolic relationships between species number and connectance are apparent in a number of collections of food webs^{3,4} though Rejmanek and Starý's data are unique in being collected by the same authors on comparable systems.

Although I agree that stability constraints make interesting predictions about food web design^{5,7}, many of which are supported by data in the real world^{4,7}, hyperbolic relationships between C and m must be considered ambiguous: a constant number of predators and prey per species might be caused by various factors other than system stability.

The webs shown in Fig. 1 of ref. 1 are quite remarkable in that they lack species that feed on more than one trophic level (omnivores). Omnivores average one per top predator in food webs dominated by vertebrates and considerably more in webs composed of insects, their parasitoids and hyperparasitoids⁴. The absence of omnivores may be a result of omitting hyperparasitoids (which are highly omnivorous), and (or) placing emphasis on the prey's predators, rather than all the prey of a particular predator. Considering only a species' predators gives source webs, in Cohen's³ terminology, considering all the prey gives sink webs. Cohen's single source web is also unusually simple^{4,7}. If omissions have been made they will reduce the connectance of the webs.

Counting interactions directly between species that share the same food supply implies competition over and above that for these shared resources. Even intraspecific interference appears unusual in insects, though data are few⁸. Adding

connections in this way will increase the apparent connectance.

Finally, I must caution the use of May's inequality for the kind of data discussed here. May's result that mC should be less than one divided by the squared interaction strength comes from the application of the semicircular law^{9,10}. Assuming a number of features which, at best, are only approximated by insect population dynamics, one obtains this result: a semicircle describes the frequency distribution of the real parts of all the eigenvalues. The semicircle is centred on the average of the self-limiting terms in the system. May assumed all the self-limiting terms to be unity and so unity appears in the numerator of the inequality. This is probably appropriate for communities composed entirely of resource limited competitors, but does not seem reasonable for food webs. Here, only species at the base of the web are likely to suffer external resource limitation¹¹, though the phenomenon of 'pseudo-interference' in insect parasitoid systems will add more self-limiting terms^{12,13}. In short, if connectance were to be restricted by stability considerations, one might expect the limit to be related to the number of externally resource limited species, and for this number to vary between systems. I thank Post *et al.*¹⁴ for a discussion on this last point.

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REJMANEK AND STARÝ REPLY—Pimm is right to suppose that the number of predators and prey per species may be independent of the number of species in the community (or, more precisely, there is a constant mean number of edges per vertex in diagrams of food webs). Unfortunately, this fact can hardly be viewed as an attribute of species *per se*. At the moment, dynamical community constraints seem to provide a more plausible explanation of why mC is constant.

In his discussion Pimm repeats in more words what we said in our last sentence¹, and we agree with him. Only the introduction of hyperparasitoids of the genus *Dendrocerus* (Hymenoptera, Ceraphronoidea) and predators of the family Syrphidae (Diptera) into our¹ plant-aphid-parasitoid food webs raises the value of mC to a figure comparable to the mean for Cohen's web collection. On the basis of data available to us², the mC product for Cohen's 'community' and 'sink' food webs exhibited mean values of 4.21 and 5.29, respectively.

Incidentally, hyperparasitoids of aphids are rarely omnivorous^{3,7}. A high proportion of omnivorous species seems to be typical of inherently unstable communities such as inhabitants of oak galls⁸.

Pimm's most important point is the question of the number of self-limited species in real food webs. According to him, diagonal elements of interaction matrices should be zero for all consumer species. The importance of the number of externally resource limited species for stability of such matrices has been stressed by Saunders⁹. We agree that self-regulation of consumer species is rather rare in nature and only scattered evidence^{10–13} supports May's¹⁴ assumption of self-regulation at all trophic levels. The constant value $a_{ii} = -1$ is, of course, artificial and has been chosen by May to set a time scale for damping time. Some increasing probability density function on the interval $(-1, 0)$ seems to be more realistic. This implies shifting the critical values for stability in May's model to lower 'tolerable' connectance. But most of the links in food web diagrams are in no way density dependent or controlling¹⁵. Then, an increasing number of species does not necessarily cause a decrease in the probability that the interaction matrix will be stable (resilient in the sense of Harrison¹⁶): the main point of our¹ letter.

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Bombesin—satiety or malaise?

It has recently been claimed by Gibbs *et al.*¹ that bombesin (BBS), like cholecystokinin (CCK), produces satiety in rats. However, their report ignores crucial evidence and lacks critical controls.

First, there is good evidence that CCK suppresses feeding because it produces malaise. This has been shown by appropriate conditioned taste aversion tests². Reports of aversive symptoms in human subjects injected with small doses of CCK corroborate the evidence from rats³. Further, doses of CCK that produce food intake suppression produce abnormal patterns of duodenal activity, quite different from the patterns observed during normally induced satiety, from which it is concluded that the amounts of CCK injected to produce intake suppression are much larger than those that are normally secreted⁴.

Second, Gibbs *et al.*¹ present no relevant evidence to show that BBS in the dose injected is not an aversive agent. It is well known^{5,6} that even quite powerful doses of some agents producing conditioned taste aversion produce no observable symptoms of distress. Moreover, only a very small dose of one such agent (LiCl) is necessary to suppress food intake to the extent reported for BBS⁷. Gibbs *et al.*¹ did not use the conditioned taste aversion test to screen for aversive effects. (In such a test a taste is followed by a dose of the agent being tested for aversive properties. If the taste is avoided in a subsequent test, this shows that the agent is indeed aversive.) It has been argued that the results of such a test are ambiguous: conditioned satiation⁸ could lead to a reduction of intake in the same way as conditioned aversion; or, satiation and mild discomfort or malaise may in fact be identical. Both such arguments are contradicted by the experimental evidence. Conditioned satiation would resemble conditioned aversion in a situation where the rat is presented only with the conditioned taste in the test (the so-called single bottle test). However, in a two bottle test where the rat chooses between a neutral solution and the conditioned solution, experiments have shown that when the proper nutrient solutions^{9,10} as well as other reinforcers¹¹ are paired with a taste then that taste is preferred over a neutral taste in a subsequent test. On the other hand, when aversive agents are paired with a taste, that taste is avoided^{12,13}.

Clearly, conditioned satiation leads to the opposite result that conditioned aversion does. Such results also dispose of the second argument. If satiation was aversive, stimuli conditioned to it would not be preferred. Even tastes paired with very weakly aversive stimuli produce an aversion⁷. Without evidence from proper behavioural screening tests, the claim that bombesin is a satiety agent cannot be taken seriously. At present it seems most probable that BBS, like CCK, is being administered in unphysiological doses and is therefore suppressing food intake by producing malaise.

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GIBBS AND SMITH REPLY—We did not use a conditioned taste aversion paradigm in our study of the effect of bombesin (BBS) on feeding¹ because this paradigm can no longer be considered a critical test of the presence or absence of malaise². As a variety of agents (including isotonic saline and chlorpromazine, a drug with anti-nausea action) which serve as effective unconditioned stimuli for the formation of conditioned taste aversions^{3,4} do not produce sickness, the conditioned taste aversion test cannot be used as evidence of sickness. Conversely, as some rapidly acting rodenticides (including strychnine and cyanide) do not produce a conditioned taste aversion⁵, the failure to produce a taste aversion is not evidence that rats are not sick⁶. Thus, sickness is neither necessary nor sufficient for the formation of a conditioned taste aversion.

We rely instead on our demonstrations that BBS fails to affect the initial rate of feeding, fails to affect body temperature, fails to affect water ingestion in the range

of doses that reduce food intake, and selectively affects feeding¹. These results are good evidence that the effect we report on feeding is not due to sickness; none of these results would have been predicted if BBS were acting simply by producing illness. We have previously reported very similar observations⁷ as indications that the action of cholecystokinin (CCK) on food intake is not due to malaise, and it is important that these observations have proved to be excellent predictors of the results of human studies.

The satiety effect of CCK in humans has been dissociated from subjective reports of discomfort in three studies to date. The first report was that of Sturdevant and Goetz⁸, which Deutsch misquotes. These authors demonstrated that, while an intravenous (i.v.) injection of impure CCK at a high dose produced side effects, an i.v. injection of a lower dose significantly reduced food intake without causing any side effects or discomfort. This critical dissociation has now been reproduced twice in humans: pure or highly purified preparations of CCK reduce food intake⁹ and ratings of appetite¹⁰ without producing reports of illness.

Deutsch concludes by assuming that CCK and BBS are producing malaise because the doses are unphysiological. We have previously shown that intraperitoneal injections of impure CCK as small as 2.5 Ivy units kg⁻¹ will reduce food intake in rats⁷ and that a slow i.v. infusion of a pure preparation of CCK as small as 30 ng kg⁻¹ will reduce food intake in humans⁹. Both doses are within the ranges required to achieve the classic visceral effects of the hormone in each species, and are therefore likely to be physiological.

The evidence does not support Deutsch's dismissal of the actions of CCK and BBS on food intake. The possibility that these peptides have a role in satiety can be taken seriously.

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Mélange in Trondheim Nappe, central Norwegian Caledonides

THE speculative conclusions of Horne¹ following his contention that mélange is present in the Trondheim Nappe of central Norway are untenable in the light of our current knowledge of this region. Three main objections may be raised.

(1) The crude 'fanning of structural surfaces' across regional strike, which is central to Horne's model, is documented in several tectonic structural studies of the central Scandinavian Caledonides as a product of the Silurian orogenic deformation, and can in no way be construed as representing a subduction-related accretionary fan-structure.

(2) Accretionary prisms of arc-trench subduction complexes are composed principally of offscraped oceanic sediments, igneous rocks and trench-fill deposits. In Horne's¹ hypothetical model (his Fig. 5) the prism embraces four tectonostratigraphic units ranging from low-grade, Ordo-Silurian volcanites and flyschoid (Köli) metasediments in the east, through high-grade Gula (Seve) migmatites and gneisses of probable Sveconorwegian or older age, and into the low-grade 'Selbusjøen mélange' in the west. An accretionary prism of such extreme age range and embracing long-transported nappes of varying origin^{4,5} is incompatible with the composition and derivation of forearc subduction complexes.

(3) Continuing studies of volcanite geochemistry (major, trace and rare earth elements) confirm earlier results^{4,6} showing that the Støren Group metabasalts are ocean-floor tholeiites. With the discovery of associated sheeted dolerite dykes and gabbro in one area⁷, the interpretation of the Støren as an ophiolite fragment⁸ is even more secure. In contrast, the Fundsjø Group contains a greater proportion of acidic extrusives, and basalt geochemistry reveals that both island arc and ocean-floor tholeiites are represented⁹. Horne's¹ diagram, showing the Støren as a magmatic arc and the Fundsjø as an 'oceanic remnant'—with the Støren and Fundsjø transposed—is, therefore, misleading.

Despite these criticisms, the presence of a mélange of latest Cambrian oceanic and trench sediments, with pre-Arenig fan-thrusting and folding, would not be out of place in this ophiolite setting, but detailed mapping would be required to ascertain its true character.

A final point, and one of major importance in a consideration of Horne's hypothesis, is that the concept of Silurian, pre-F₁ obduction⁴ has been discarded. Based on analysis of new data from mapping and structural studies, the Støren-Fundsjø obduction, eastward on Gula rocks, is now considered to date to pre-Middle Arenig time^{8,10}, of Finnmarkian/Grampian age¹¹, with the

Lower Hovin deposited on the folded and uplifted ophiolite fragment^{8,12,13}. This situation is comparable with that in the Lower Palaeozoic of western Norway^{8,10,11}.

Continuing southeastward subduction during Ordovician time, although perhaps located further to the west, produced an Arenig-Llanvirn volcanic arc and marginal basin spreading¹²⁻¹⁴. Horne's¹ interpretations, therefore, conflict with the results of other recent research in the Trondheim region Caledonides⁷⁻¹⁴.

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HORNE REPLIES—Roberts' objections to my speculative model for the early development of the Trondheim Nappe do not bear directly on the tenability or applicability of my hypothesis. My brief response to each criticism follows.

(1) Roberts' structural arguments are debatable, yet he offers no evidence to support his contention. Clearly, an inherited fan structure would have been reactivated during and after obduction as the Trondheim Nappe was translated eastward during the Silurian. The problem involves dating the initial deformation and identifying the cause of earliest thrusting. I interpret Roberts' F₁ deformation¹ as occurring synchronously with the accretion process.

(2) Roberts' second criticism contains several inaccuracies. My Fig. 5 (ref. 2) shows that, although I presumed that the Seve and Köli sequences had been bulldozed in front of and obducted with the Trondheim Nappe, I did not consider them to have been incorporated into the forearc region by sea-floor accretion.

Comprehensive and comparative studies of forearc regions³ show that subduction complexes are indeed characterized by heterogeneous stratal assemblages that include various igneous rocks, metamorphic tectonites, chaotic mélanges, and isoclinally folded sequences of bedded sediments that "represent a wide range of oceanic environments" (p. 19, ref. 3). Roberts' supposition that the Gula Group is Precambrian is based on a tenuous correlation with tectonites exposed to the west of the main Trondheim succession that have only been indirectly implicated to be Precambrian⁴.

(3) The statement that Støren metavolcanics are ocean-floor tholeiites is misleading. They are spilitic greenstones that have suffered both regional metamorphism and local metasomatic alteration. The presence of associated bedded pyroclastics, including lappili tuff and limestone, certainly argues against a deep marine environment. Notwithstanding recent evidence⁵ from modern sea-floor basalts that warns strongly against using trace element composition for tectonic labelling, Roberts has used the trace element distribution within Støren greenstones to ascribe the Støren to either ocean floor or arc settings⁶. Intra-ocean arcs clearly must be built on and from the oceanic lithosphere, and many ensimatic arcs tend to nucleate along transform faults with attendant ophiolite emplacement⁷. The infrastructure of such arcs should indeed comprise ophiolite fragments⁸, as Roberts testifies.

Roberts' final point concerning the Hovin having been deposited unconformably on folded Støren metavolcanics contradicts well documented field evidence from many areas in the Trondheim region. I agree that the age evidence bearing on time of obduction is crucial, and that it demands critical analysis.

Note added in proof: Very recent evidence from Troms⁹ casts doubt on the concept of pre-Arenig Sinnmarkian abduction.

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Exploring pictures by hand

RECENTLY, Magee and Kennedy¹ have found that blindfolded sighted people can identify a raised-line drawing more readily when the index finger of one hand is guided along its outline than when the finger is allowed to trace the figure without guidance. This finding could evidently be used in perfecting methods of teaching the blind how to use and interpret raised-line drawings.

However, three difficulties render the conclusions of this study inapplicable to the teaching of blind persons. First, the blindfolded condition is in no sense equivalent to, or a model for, blindness. The study should have been conducted both with blind children and with recently blinded adults, who may differ from each other and from the sighted population in the extent of their first-hand experience with objects whose two-dimensional representations they are asked to identify tactually.

Second, and much more important, a blind person does not perceive a raised-line drawing by tracing its outline with the tip of one index finger; instead, the drawing is first scanned by several fingers of both hands, and later traced in segments and in various directions by the index fingers of each hand. This method is analogous to visual inspection of pictures. If a raised-line illustration consists of more than just an outline, the single-finger tracing method would be inefficient, confusing and uninformative.

Finally, the results that Magee and Kennedy obtained probably depend on the size of the drawing. From their data I calculate that it took an observer about 30 s to be guided around the perimeter of a swan whose representation fits in a 10-cm square. I suspect that single-finger tracing without guidance is far more efficient on a small drawing than on one as large as that used by Magee and Kennedy. In fact, moderately small-scale drawings are probably easier to interpret than large-scale illustrations, as was

clearly recognized by those who developed and perfected the Braille alphabet.

In conclusion, the work of Magee and Kennedy is one of many examples of the tendency for research on blindness to be conducted with little understanding of blindness itself. Despite this ignorance, conclusions from such studies have great impact on the education of the blind.

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MAGEE AND KENNEDY REPLY—Vermeij's response to our report¹ is concerned with the practical application of our findings to the use of raised-line drawings by the blind. This is a fruitful approach to research in this area. Yet, one should not confuse an empirical method used to test a theory with an extrapolation to the pedagogy of the blind.

We tested the generality of a well established claim in haptic form perception—namely that unaided exploration is superior to aided exploration. Contrary to popular opinion, we found that self-guided exploration of raised-line drawings is inferior to aided exploration. We then used this result to evaluate the relative contribution of cutaneous and kinaesthetic sensitivity to identification and found that kinaesthetic information is predominant.

Vermeij does not question these basic findings but does ask whether they are applicable to the blind. It should be noted that traditionally (see, for example, ref. 2) the blindfolded, not the blind, have been used as subjects in experiments on haptic form perception. To the extent that the blind and blindfolded have that same physiological

mechanisms underlying the receptive functions of the hand, we believe our results to be generally applicable. Indeed, Kennedy and Fox³ have found very little difference among the blindfolded, adventitiously blind and congenitally blind in their ability to identify raised-line drawings.

Vermeij says—and we agree—that a blind person generally uses more than one finger when exploring a raised-line drawing. However, the crucial point is that a technique focusing on a single finger allows one to manipulate exploration and make comparisons in performance which would be exceedingly difficult, if not impossible, if 10 fingers had to be controlled simultaneously. Moreover, the blind are able to identify drawings with a single finger. Note, we are not proposing that single-finger tracing is optimal or establishing the efficacy of this mode of exploration compared to other strategies.

Vermeij speculates that our results probably depend on the size and complexity of the drawing. Our pilot studies have not found any interactions of this type.

In sum, our major theoretical findings are quite clear. Guidance can be useful in haptic form perception and the source of information subserving identification of raised lines is predominantly kinaesthetic. The practical implications are that a teacher need not refrain from helping a blind student explore raised-line figures and that an emphasis on movement variables may be beneficial when displays of this type are made.

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BOOK REVIEWS

The ethological approach to human behaviour

Stuart Sutherland

THERE are two distinct ways of explaining behaviour. One is to show what function it serves, the other is to specify the mechanisms that produce it. Ethologists have concentrated on the former kind of explanation, psychologists on the latter. If one is interested in function, it is essential to observe the animal's responses in its natural environment; only by recording the occasions on which a given kind of behaviour occurs and taking note of the context can its likely function be assessed. Ethologists have therefore concentrated on observations of naturalistic behaviour, whereas psychologists have tried to arrive at the underlying mechanisms by experimentally manipulating behaviour in the laboratory. The dichotomy between the two disciplines has of course never been complete: Niko Tinbergen, one of the doyens of ethology, has performed many ingenious experiments, and from J.B. Watson onwards psychologists have increasingly come to apply ethological methods to the study of human beings, particularly to ontogenetic development and to social interactions. *Human Ethology* is based on the proceedings of a conference the aim of which was to assess both the merits and the difficulties of this approach.

As so often happens, most of the contributors have failed to keep their eye on the ball and have merely provided detailed accounts of their own researches. No general message emerges, though some striking if unsystematized new findings are described. For example, it would appear that the behaviour of mothers towards their infants is nicely adjusted to the infant's stage of development. A mother tends to hold her new-born baby about 25 cm in front of her face: this distance is that at which objects are brought into sharpest focus for the baby, though not necessarily for the mother. Again, the repetitive nature of the baby-talk in which infants are addressed may be suited to the limited rate at which they can learn and its high pitch may match that of their own voices. Yet it is not known whether making

Human Ethology: Claims and Limits of a New Discipline. Edited by M. von Cranach, K. Foppa, W. Lепенies and D. Ploog. Pp.764. (Cambridge University Press: Cambridge, UK, 1980.) Hardback £35; paperback £12.50.

baby-talk is an inborn skill or whether it is culturally determined, and without experimental manipulation we can only speculate about how necessary it is for the baby to be exposed to this form of talk. Very young babies have a remarkable capacity for imitation: they can even respond in kind to seeing their mother stick her tongue out. It seems possible that the capacity for this sort of imitation is inborn, but what sort of genetically determined mechanism is needed for the baby to match up its mother's tongue to its own which it has never seen?

The virtue of the ethological approach is that it provides many new data that are a challenge to explanation. Nevertheless, as several contributors to this volume, and in particular William Mason, point out, it has its dangers. Ascribing a function to behaviour is often a perilous undertaking: Freud relied wholly on functional explanations and many of them are virtually untestable. Mason reports a study of infant monkeys reared by canine mothers: their development was sufficiently normal for him to conclude that "the relevant dimensions of the early social environment for the development of coping strategies are not closely tied to the species-specific structure of the mother-infant relationship". Moreover, there are well-attested cases of infants reared under conditions of appalling deprivation who on being transferred to a better environment developed into normal adults. Despite the volume of research currently being conducted on the subject, we can hardly begin to specify the range of experience that is necessary for normal development.

Not all of *Human Ethology* is devoted to

ontogeny; amongst the other topics considered are rituals, attitudes to property and territory, aggression, and other aspects of social relationships. The fallacious argument that if there is an analogy between an aspect of human behaviour and that of other animals, the behaviour must be genetically determined, appears repeatedly. To the extent that learning itself depends on genetically determined structures, all behaviour has a genetic component, but to say of a given behaviour that it is genetically determined is an empty statement unless the range of environmental experiences necessary for its occurrence can be specified. We still know remarkably little about the malleability of human behaviour or human emotions, notwithstanding the pronouncements of the sociobiologists.

Human Ethology contains contributions by ethologists, social psychologists, psycholinguists, anthropologists and sociologists. The debates between them have an air of unreality largely because, despite the interest of the observations recorded, few contributors put forward properly articulated theories. Unfortunately verbiage is catching and the sociologists appear to have infected the other contributors with their own brand of pompous obscurity. One chapter begins: "In this paper I claim evidence that human infants are *intentional, conscious and personal*; that above all they have a faculty of *intersubjectivity*" (p.530). Who could argue with that? Or again, what is one to make of the claim that "privacy operates as a dialectic, optimizing, multilevel boundary process" (p.101)? Even the author of this rebarbative phrase has doubts, since he goes on "The question arises: So what?" It is a question that might well be asked of the whole book. □

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Earth science

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The Earth: Its Origin, Structure and Evolution. Edited by M.W. McElhinny. Pp.597. (Academic: London and New York, 1979.) £36, \$83.

THIS volume is a collection of 17 review papers written to honour the two outstanding geophysicists who were responsible for the development of earth sciences at the Australian National University in Canberra. Under the direction of John C. Jaeger and Anton Hales, the ANU has become a world centre of earth-science research. This book, written exclusively by present and past members of the Research School of Earth Sciences, is one of the most significant collections of papers on this vast subject to appear in some years. The 'subject' is the Earth, and the material is about equally divided between chemistry and physics. There are sections on the crust, the mantle and the dynamics of the core.

The crustal chapters include petrogenesis of oceanic crust, evolution of granitoids, and the relation of porphyry copper deposits to calc-alkaline volcanism. The power of isotopes and incompatible trace elements is fully exploited in chapters by Taylor, Compston and Chappell. The chapter by Green, Hibberson and Jaques withdraws the previous Canberra model for the origin of mid-ocean ridge basalts, the basis of the pyrolite model, in favour of the O'Hara model which had formerly been bitterly contested. Green *et al.* now prefer a picritic parent magma and conclude that there cannot be a simple direct genetic relationship between the mafic and ultramafic rocks of ophiolite complexes. This important conclusion, if verified, will make it necessary to reconsider the composition of the upper mantle.

There are four chapters covering the areas of geomagnetism, palaeogeomagnetism, polarity time-scales and apparent polar wandering, and one on the history of the Earth's rotation. Altogether there are 11 chapters with primary emphasis on geophysics.

The mantle receives a great deal of attention, but much of the discussion of composition and evolution is based on concepts developed before the constraints provided by isotopes, the incompatible trace elements and anelasticity were fully appreciated. Subjects covered include composition, structure, phase changes and elasticity. The chapter on mantle convection by Stevenson and Turner is one of the best of several recent reviews of the subject.

The chapter by Liu summarizes the large amount of information he has obtained in

diamond anvils on phase changes in mantle silicates. This productive, but exploratory, field of research has verified many of Ringwood's predictions about the crystal structure of various regions of the mantle. It is still uncertain at what depth these phase changes occur and what the physical properties, other than density, will be. For this, a large amount of *in situ* data and better controlled temperatures will be required. Liu has made a preliminary attempt to estimate density as a function of depth in various mantle mineral assemblages. One interesting point is that eclogite is denser than garnet-peridotite to a depth of about 500 km but is less dense between 500 km and 800 km. This seems to preclude subduction of oceanic lithosphere into the lower mantle. If verified, this will have enormous implications for the composition and evolution of the mantle and convection therein. Ringwood's chapter on the composition and evolution of the Earth develops the viewpoint that

the mantle is basically chondritic and homogeneous.

The book is rounded out with chapters on rock mechanics, earthquakes and plate tectonics.

The breadth of coverage from the faculty and alumni of a single institution is tribute to the wisdom and foresight of Jaeger and Hales who always managed to keep ANU at the forefront of earth science by picking the areas and people that would contribute most to the understanding of our planet. This volume is an important contribution to that end. It is probably the most comprehensive single volume on the subject of the origin, evolution and dynamics of the Earth presently available. It is unfortunate that its price will make it unavailable to students and many libraries.

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Archaeological dating

Georges Valladas

Thermoluminescence Techniques in Archaeology. By Stuart Fleming. Pp.233. (Clarendon/Oxford University Press: Oxford, 1980.) £15.50.

THE technique of dating by thermoluminescence (TL), a method which has been well established now for some dozen years, is discussed in articles scattered among many specialized journals. This book will therefore be welcome to physicists in that it gathers together the principles of the subject in one place.

The fundamentals of TL were developed in the Research Laboratory for Archaeology and History of Art at Oxford under the leadership of Martin Aitken. The author of this volume, Stuart Fleming, is one of the team of physicists trained in the laboratory at Oxford, and is thus well placed to have written this work, the fruit of experience acquired in the course of the investigations which were to provide the first absolute ages of ancient pottery.

Certain minerals making up a piece of pottery emit light when they are heated, and the older the pottery the more intense the light. The phenomenon is essentially due to the action of the radioactivity contained in the minerals. This action can be represented as the dose of radiation received by the minerals in question over the time elapsed since the original baking of the pottery, an act which destroys its initial (potential) TL. Thus the age of a piece of pottery can be deduced if the dose received in a single year is known. The book treats the following three, more or less

interdependent, subjects: (1) the preparation of the mineral after its extraction from the pottery; (2) the use of thermoluminescence to define the radiation dose received by the mineral — the archaeological dose; (3) the analysis of the radioactivity of the pottery and of the surrounding earth in order to deduce the annual radiation dose to which the mineral has been subject.

The preliminary pages are dedicated to crystalline TL and its measurement, and the properties of natural radioelements (uranium, thorium, potassium-40) and the dosimetry of α -, β - and γ -rays are the objects of a detailed analysis. The author examines the consequences of the structure of ceramics on the distribution of the radiation dose among its constituents. The radioelements are concentrated in the clay phase of the pottery and are practically absent from the crystalline inclusions. Among the latter, those which are of a certain size and occur frequently in ancient pottery, such as quartz grains, therefore receive a weaker dose than the clay fraction. Hence, α -rays penetrate to few of these grains which thus receive doses of nothing but β - and γ -radiation. This is not the case for micro-inclusions, for which the α -dose is important. Now, the TL induced in each circumstance has distinct properties. These facts lie at the root of the two principal methods of dating: the 'quartz-inclusion technique' (S. Fleming) and the 'fine-grain technique' (D.W. Zimmerman).

In Chapter 3, the quartz inclusion technique is discussed. Fleming analyses the properties of the TL of quartz and defines that interval of temperature best adapted to the determination of the archaeological dose. This is a method of great importance due to the reliability of

quartz as a dosimeter and the relative simplicity of the calculation of the annual dose. Fleming is primarily interested in pottery and does not consider the natural extension of this technique to the heated stones of prehistoric hearths (flint, quartzites, sandstone etc.), a method still in its infancy when the book was written.

Also in Chapter 3 there is a résumé of the elegant method which uses a very sensitive thermoluminescence dosimeter (fluorine) to measure directly the annual dose deposited by β -radiation in pottery. This method is also applicable to the measurement of the γ -dose coming from the soil. Here the problem of radon (radium emanation) must be considered, the active deposit of which is responsible for about 50% of the γ -dose, which therefore depends on the movement of radon in the soil. Chapters 2 and 6 give a very complete analysis of this problem.

The pre-dose technique (Chapter 5), which also involves quartz inclusions, has a use limited to those ceramics which have received a relatively low archaeological dose and are thus relatively young (< 1000 years old). On the other hand this permits it to be used instead of carbon-14 techniques which are practically useless for any period more recent than the fourteenth century.

Despite the difficulties which are inherent in it and which concern in particular the contribution of α -radiation

to the annual dose, the fine-grain technique has been brought up to the level of a routine measurement at Oxford. It is the subject of a detailed exposition in Chapter 4 which begins with the art of making the deposits of fine grains on aluminium discs which forms the basis of the whole method.

The last third of the book and Appendix E constitute an anthology of dates and of applications of TL to the authentication of ceramic artworks and of those bronzes which contain kernels of baked clay. Results bearing on the neolithic ceramics of Central Europe and Africa, relative to the Nok culture among others, are examples showing the great importance of TL for the dating of sites which in the absence of organic remains are not datable by the carbon-14 method. When the two methods have been applied simultaneously, the ages they have yielded are in agreement.

In conclusion, the methodological exposition is as complete as can reasonably be wished, given the present state of TL dating. Moreover, the book is rich in examples. Novices will find encouragement here to face up to problems which are still insufficiently resolved and archaeologists (particularly those strong in maths!) will discover the lucid explanations of the technique they have lacked until now. □

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Large African wildlife

Brian Bertram

The Ecology and Conservation of Large African Mammals. By S.K. Eltringham. Pp.268. (Macmillan: London, 1980.) £15.

WE have waited a long time for this book — not only because Dr Eltringham has taken a long time writing it but also because there has been a conspicuous and lamentable absence of a book which spanned the wide and fascinating area of field studies of large African mammals. The author is thoroughly well qualified to fill the void. He was Director of the Nuffield Unit of Tropical Animal Ecology in Uganda, and has for years been Associate Editor of the *East African Wildlife Journal* (now the *African Journal of Ecology*). It is this journal which has published many of the results of the large mammal field studies conducted at the Nuffield Unit, the Serengeti Research Institute, the Tsavo Research Project and East African universities.

So Keith Eltringham knows his facts. What has he done with them that is new? Essentially he brings them together in one place, and he discusses in general terms not the various mammal species themselves but the differences between those species, the interactions among them, and human interaction with and influence upon them. The book thus complements the many excellent and detailed accounts, at both popular and scientific levels, of field studies of single species or groups. It is not a review of those accounts — rather it provides the background to them, plus a much needed dose of general knowledge, experience and common sense, which may be widely shared but is too rarely written down.

The book starts with a brief summary of the African regions, and a description of some of the techniques used in wildlife research, principally counting, marking and immobilizing methods. Each of the following six chapters takes a particular theme and considers the range of ways in which large African mammals behave in that respect. These themes are social structure and social behaviour, followed by territorial behaviour, then population ecology, reproduction, herbivore feeding and carnivore feeding. Three chapters on conservation, wildlife management, and Man and national parks complete the text. To my mind the last six chapters are much better than the four preceding ones. There is a valuable bibliography of over 400 references, maps of African national parks and reserves, and a useful list of scientific names of mammals each with its authority and date. There are 27 informative black and white photographs, about 20 tables and as many diagrams.

A range of lattice path problems

Michael Berry

Lattice Path Counting and Applications. By Sri Gopal Mohanty. Pp.185.(Academic: New York and London, 1980.) \$24, £13.60.

CONSIDER the unit square lattice whose points have integer coordinates x, y , and let a 'path' be a sequence of steps between neighbouring points. How many paths run from the origin to the point with coordinates mn , ($m \neq n$) without touching the line $x=y$? It is with such problems, and their applications, that this book is concerned.

In the general case, where the lattice has more than two dimensions, where the paths are subject to more complicated restrictions (such as not touching boundaries of general form), and where diagonal steps are permitted, the enumeration of paths is very difficult. In many cases, however, recently developed combinatorial techniques make it possible to obtain solutions in closed form.

It is remarkable what a wide range of problems can be put into correspondence with the enumeration of lattice paths. The classical example, due to Bertrand, is to calculate the probability that the victor in

an election had more votes than the loser at every stage of counting. Another case is random walks on a line, for example the probability distribution of times when the walk (starting from the origin) first passes a given point. Other problems considered in the book are the lengths of queues, the enumeration of Cayley trees and the convolution identities (of binomial type) that can be proved by considering combinations of lattice paths.

For all problems treated, the exact solutions are given, involving for the most part binomial coefficients. In many cases, however, what are required are asymptotic approximations (for example as the number of steps tends to infinity), which are simpler and might be derived directly; but no such results are given here.

This is not an easy book to read. The author's style is terse, and the non-experts for whom the book is presumably intended will have to work hard to follow the argument. Such phrases as "one can easily show that" or "a little reflection will show" are unhelpful, and an argument alleged to provide "pictorial insight" would have been clearer if accompanied by an illustration. However, the book is greatly improved by the provision of extensive lists of references and challenging problems at the end of each chapter. □

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What are the merits and limitations of the book? Most importantly it fills a glaring gap. It gives a range of useful information on a range of interesting species, and it makes it palatable — the text is free of jargon yet does not underestimate the reader's intelligence, and the reader is certainly going to need intelligence. The arrangement allows, indeed causes, abundant discussion of general topics such as social hierarchies, functions of territoriality, population regulation, socio-biology, plants' defences against being eaten and their use of animals in seed dispersal, the range of herbivores' anti-predator techniques, 'the elephant problem' (to cull or not to cull), fire, disease and tourism. Evolutionary and functional questions abound.

It is extremely difficult when dealing with diverse, often 'hot', subjects to trace a satisfactory route which avoids oversimplifying, confusing, pontificating or taking sides, but in most cases Dr Eltringham manages to do so. Of course, he says things which to my mind are highly contentious, such as (p.231) that "If disease is identified in a national park attempts should be made to eliminate it" (this also conflicts with p.228 which states that "Disease is a natural factor in wild animals and rarely requires management attention"). I also disagree with his statement (p.210) that "It is questionable whether reintroduction will ever become a practical proposition, although much play is made by zoos and other animal collectors on their important conservation role in preserving species for eventual release". After all, it is not "questionable" that reintroduction of the European bison has already proved to be a practical proposition, and indeed a success. Zoos recognize better than most people the variety of problems involved, but we do not accept that they are insuperable.

I would do more than disagree with statements such as (p.41) "a pack of wild dogs, a herd of buffaloes and a troop of baboons are all essentially similar in social structure and should be given the same collective name", since wild dogs, unlike the others, have a most unusual system involving female transfer and usually only one reproductive female per pack. Similarly (p. 109) "in the llama the process [of copulation] occurs with both partners lying down"; the mind boggles — in fact the male squats on top of the female who rests on her brisket. But who could produce a stimulating book without the occasional contentious or fallacious statement? And who could fail to agree with so lovely a sentence as (p.122) "Fortunately for the mother [rhino] the baby is born without its horns"?

The book is largely intended for biology students in universities, as an introductory textbook to more detailed studies. It will inform the non-biological visitor to Africa, but he or she will have to work hard at it. And it provides a useful source of

references, nearly a third of them from the *East African Wildlife Journal*.

Despite its title, the book is mainly about the large mammals of the East African grasslands only, but is none the worse for that. It ignores, for example, species such as the okapi, pigmy hippo, sable, greater kudu and chimpanzee, on some of which a considerable amount of field research has been carried out. But it is right, I think, to emphasize the East African side, because

East Africa has on the whole produced the most, as well as the best, field research on large mammals. Directly or indirectly, Keith Eltringham has been associated with a great deal of it. And directly or indirectly his fine book will stimulate and help much more of it. □

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Plasma phenomena and the Solar System

S.-I. Akasofu

Solar System Plasma Physics. Edited by Charles F. Kennel, Louis J. Lanzerotti and Eugene N. Parker. Three volumes. (North-Holland: Amsterdam and New York, 1979.) Vol. I. *Solar and Solar Wind Plasma Physics*, pp.340. Vol. II. *Magnetospheres*, pp.396. Vol. III. *Solar System Plasma Processes*, pp.373. Each volume Dfl.150, \$73.25; three-volume set Dfl.382, \$186.25.

IONIZED gases have been a focus of intense study in several subject areas — plasma physics, controlled nuclear fusion and gaseous discharge research, astrophysics and geophysics. From several sub-disciplines in astrophysics and geophysics, such as solar physics, geomagnetism, magnetospheric physics, cosmic ray physics and ionospheric physics, has emerged a new discipline, solar system plasma physics. This is defined by the three editors of the book as "the study of plasma phenomena throughout the solar system, extending from the solar photosphere to the outer boundary of the heliosphere (the cavity formed in the interstellar medium by the solar wind)".

The beginning of this new discipline can be traced back to the pioneering work by Sydney Chapman, Hannes Alfvén and others on geomagnetic storms. It is, however, only in the last two decades that it has been developed explosively, stimulated by the availability of *in situ* spacecraft observations. In fact, the book "celebrates the twentieth anniversary of space research", providing ample evidence of spectacular progress and establishing an important milestone in this particular field of science.

The first volume deals mainly with solar activity, the solar wind and cosmic rays. The second volume is concerned with the Earth's magnetosphere and ionosphere and the planetary magnetosphere. The third volume singles out several specific plasma processes which may be seen throughout the Solar System, such as

collisionless shock waves, magnetic field reconnection, hydromagnetic waves, plasma waves and plasma instabilities. These subjects are reviewed in 24 papers, contributed by 28 authors. Obviously, this book is a historic undertaking.

Although the level of the papers varies somewhat, new graduate students should be able to grasp the general scope of this area of research since many of the articles are written in a textbook style, and there should not be any difficulty for plasma physicists to review the progress which has been made in space plasma physics. Astrophysicists, too, should find the book very useful, since many papers are based on *in situ* measurements of plasma quantities and of magnetic fields. Similar conditions may be present in some stellar atmospheres and interstellar space. This is likely to be the case because a magnetosphere appears to be a rather common feature in various astrophysical phenomena (the head-tail galaxies, pulsars, magnetic stars etc.).

Above all, specialists in the discipline will, undoubtedly, find the three volumes most valuable. In the ever-increasing trend towards specialization, it is worthwhile to read such a series of reviews which covers practically the entire field. It provides a unique opportunity for the specialist to familiarize himself with major progress and the current ideas in each subdiscipline. Such a comprehensive edition of review papers covering the entire discipline will not appear too often. All the papers are extensively and properly referenced, and the readers should not have any difficulty in expanding their knowledge. It appears that all of the contributors made a special effort to provide a good reference list.

Each author attempted to describe unsolved problems and difficulties which are facing his particular subdiscipline: for example, T.E. Holzer presents a "Timetable for Solar Wind Research"; B.U.Ö. Sonnerup gives a useful list of recommendations for future study of reconnection processes; and astrophysicists may particularly be interested in "Recommendations for Continued Plasma Wave Research" by S.D. Shawhan. I feel that more emphasis along these lines would have made most of the articles more stimulating. In most papers discussion of current ideas predominates, although some of the problems in the field appear to be insur-

mountable without new approaches.

In his review of the modulation theory of cosmic rays, L.A. Fisk describes the present situation as "in a state of wary optimism". Although this may indeed be the case in certain areas, it remains to be seen how many of the ideas will survive another decade. It is useful to note in this connection that one of the most important events in solar system plasma physics in the last few years was the finding of a very interesting electric potential structure above the aurora (described in Vol. III, 1.5 and 1.6). The possibility of the presence of an electric field along magnetic

field lines in a collisionless plasma should receive wide attention by astrophysicists in general. In this connection it may be recalled that various exotic acceleration mechanisms of energetic particles have been described in the past by assuming that such a parallel electric field cannot exist. This unexpected finding of the potential structure has already considerably changed the course of our thinking and will continue to do so during the next decade. New findings by deep space probes may also drastically alter our ideas on the heliosphere. In this respect, the authors could have been a little more bold by

injecting new ideas as it is rather difficult to do so in a paper for a regular journal these days, given the conservative attitude of many referees.

As stressed earlier, however, there is no other up-to-date and comprehensive review available which covers the entire scope of solar system plasma physics. It is hoped that the book will be widely read by everyone who is interested in plasma physics in general. □

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Photoresearch for physical chemists

D.W. Turner

Photoabsorption, Photoionization and Photoelectron Spectroscopy. By J. Berkowitz. Pp. 469. (Academic: New York and London, 1979.) \$61, £39.80.

THE story of the exploration of that area of the electromagnetic spectrum which falls between the optical and the X-ray regions is an extremely exciting one, partly in terms of the succession of new theoretical and interpretative problems that have arisen, but most especially because of the elegant, painstaking and sometimes heroic experimental techniques which have been called for. From the earliest pioneering work of Schumann and Lyman to the present day, advance has always been associated with new techniques in the hands of gifted experimenters. Some major leaps forward and acceleration in activity in the area can be identified in retrospect — the observation of molecular Rydberg series in the 1930s, the application of photoionization threshold measurements in the 1950s and photoelectron spectroscopy in the following decade. The latter gave, for the first time, access to the inner valence shells of complex molecules and at this stage a unification with parallel developments in the soft X-ray region established an area of work which had become essentially chemical in its applications, though the methods were those of the physics laboratory. The present text is the first which has made a serious attempt to draw all of these threads together and show the application of these and other related techniques to the understanding of the structure of some quite complex molecules and their ions.

After a survey of some fundamental principles and an outline of physical processes underlying the absorption of high-energy radiation and ionization processes generally, the author devotes three chapters to describing photo-

absorption below the first ionization limit, quasi-discrete states above the first ionization limit and the physics of the ionization continua. There then follows a discussion of photoabsorption and photoionization cross-sections for selected molecules in which all the information available for each case is brought together for critical evaluation, and then a similar critical discussion of partial cross-sections of a range of molecules in which extensive use is made of photoelectron spectral data. A separate chapter considers the problem of the angular distribution of photoelectrons.

This is essentially theoretical with a rather small number of applications to simple molecules being described. The book ends with a survey on instruments and methods which is particularly well done.

This is a text produced to an extremely high standard and the author speaks with authority and clarity. It is to be recommended particularly to research workers in the field. Chemical physicists generally will find it a valuable reference text. □

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Principles of gel permeation

J.V. Dawkins

Modern Size-exclusion Liquid Chromatography: Practice of Gel Permeation and Gel Filtration Chromatography. By W.W. Yau, J.J. Kirkland and D.D. Bly. Pp.476. (Wiley: New York and Chichester, UK, 1979.) \$36.60, £17.45.

STUDIES of the chromatographic fractionation of macromolecules by a size-exclusion mechanism have progressed along two parallel paths. Gel filtration, which is widely used for analytical and preparative separations of biological macromolecules in aqueous media, resulted from the preparation of soft porous gels, as described by Porath and Flodin in *Nature* (183, 1657–1659; 1959). Rigid porous packings such as cross-linked polystyrene, silica and glass particles for fractionations of synthetic polymers in organic media by the technique known as gel permeation chromatography were reported in 1964–1967. The theme bringing the two techniques together in this book is the use of rigid microparticulate packings for performing fast (minutes) high-resolution separations. The decrease in the

separation time from several hours in the classical size exclusion techniques has followed from the amazing advances, to which Kirkland has made important contributions, that have occurred in liquid chromatography during the past decade. Whilst high-speed separations of many synthetic polymers may be performed with porous cross-linked polystyrene microspheres, suitable microspherical rigid packings for widespread routine use in gel filtration are still in an active state of development.

Following a brief introductory chapter, there are three chapters on retention mechanisms, band-broadening and resolution. The authors have concentrated on principles rather than detailed theoretical treatments, so the practitioner can clearly identify the important variables which determine separation power and column performance. Chapters 5–11 are the most valuable sections of the book with comprehensive accounts of equipment and detectors, column packings and technology, operating variables, laboratory techniques, calibration, data handling and special techniques. The experimental aspects are well illustrated with figures and detailed tables, with more examples taken from gel permeation than from gel filtration, but the bibliography is very selective with the total number of cited references for these seven chapters below 200. Thus, it is surprising that the

important calibration method proposed by Frank, Ward and Williams in 1968 (*J. Polymer Sci. Part A-2; Polymer Phys.* 6, 1357-1369) is not mentioned. Chapter 12 covers gel permeation applications and it is not possible in 37 pages to give a representative survey from the extensive published literature. The examples chosen are only covered briefly and the treatments of branched polymers and copolymers are

disappointing. The final chapter reviews the advances made in the last five years in high-performance separations of biopolymers with microparticulate packings.

In summary, this book is highly recommended. It will be welcomed by practitioners of gel permeation separations who have never had access to a suitable reference book. For those interested in separations of biopolymers, the authors

clearly demonstrate the advantages of rigid microspheres over soft gels for fast analytical separations. The high-performance technique is likely to be increasingly used by biochemists during the next few years as column packings are improved. □

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Attraction to viroid research

William McClements

Viroids and Viroid Diseases. By T.O. Diener. Pp. 252. (Wiley: New York and Chichester, UK, 1979.) \$30.55, £14.55.

Viroids and Viroid Diseases is a comprehensive review of these smallest of known pathogens. It begins in 1922 with the first report of the potato spindle tuber disease and moves, in the author's words, "from the field through the greenhouse and into the laboratory". Thus, we are introduced to such plant maladies as chrysanthemum stunt disease and the exotic sounding cadang-cadang disease of coconuts. However, once out of the field, potato spindle tuber viroid (PSTV) receives greatest attention and serves as a model for viroids in general. Many of the author's early experiments with PSTV are reiterated here and provide the reader with the requisite background to appreciate the critical moment in this research: the recognition, by the author, of the novel character of viroids. It was the demonstration that the causative agent in potato spindle tuber disease was an unencapsidated RNA of remarkably low molecular weight (~100,000 daltons) that led Diener, in 1971, to propose the term 'viroid' for this new class of pathogen. Subsequent work by several groups showed viroids to be extensively base-paired, single-stranded RNA existing in both circular and linear forms. This work culminated with the report of the complete sequence of the 359 nucleotide circular form of PSTV. Some studies on other viroids are also included, mainly as confirmation of PSTV results.

In light of this unusual structure, the initial confusion about the size and shape of viroids is understandable: a confusion compounded by the reluctance of some to accept a totally new form of pathogen. Much of what is known about viroid structure today is a direct result of Diener's persistence during the early stages of viroid research. This persistence is reflected in the initial PSTV studies included in this volume. Also reflected in the language of the text is the sometimes contentious com-

petition in this field.

The biology of viroids is far less well known than their structure. They do not appear to encode polypeptides; replication probably occurs in the nucleus and is host dependent. Evidence supporting both RNA- and DNA-directed replication is presented along with speculative models. It also appears that some viroid-related sequences occur in uninfected plant genomes. Even less is known about the mechanism of pathogenicity, as is illustrated by the fact that this section occupies less than one page of text. In the absence of

any demonstrated viral functions other than replication, it is speculated that viroids act at a regulatory level; however, there is no direct evidence for this. Perhaps it is this very ignorance which will fulfil the desire expressed in the preface, that this book might attract additional investigators to viroid research. Certainly, *Viroids and Viroid Diseases* is a fine starting point for newcomers. □

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Cycling bacteria

John Postgate

Bacteria and Mineral Cycling. By T. Fenchel and T.H. Blackburn. Pp.225. (Academic: London and New York, 1979.) £14.60, \$34.

MICROBES are of fundamental importance for the persistence of living things on this planet. Their transcendent role in the biosphere becomes clear when scientists consider the natural cycles of the biological elements and examine the roles of microbes at each step: the cycles of carbon, hydrogen, oxygen, nitrogen and sulphur are utterly dependent on the chemical activities of microbes. This truism, well known to microbiologists, is at last penetrating to non-microbiological ecologists and environmentalists and is indeed being recognized in the activities of international agencies such as SCOPE. So a text that provides a background in terms of the ecology and chemistry of microorganisms is very timely. The authors have made distinguished contributions to microbial ecology and their expertise is well displayed in the central chapters of the book, which concern bacteria in detritus food chains (Chapter 3), the carbon cycle (Chapter 4), the nitrogen cycle (Chapter 5), and the sulphur cycle (Chapter 6); cycles of other elements are dealt with in Chapter 7 and a global view of the major cycles is presented in the final article, Chapter 9. They have deliberately chosen to exclude eukaryotic microbes from their presentation, as well as most industrial and

economic aspects of their topic, so some imbalance must be accepted.

The authors' subject (unrelated to mineralogy, by the way) is vast so their treatment of it in what amounts to about 130 pages of core text is amazingly condensed. While brevity is a virtue, it can also be misleading if authors make categorical statements over issues which many would consider controversial or unsettled: examples of questionable items here include the evolutionary age of biological nitrogen, the unimportance of nitrification in the nitrogen cycle, the ability of *Desulfovibrio* to oxidize hydrocarbons, the reliability of S-isotope fractionation in geomicrobiology. Brevity may also have led to some factual errors and use of curiosities of nomenclature, but in compensation there are excellent sections dealing with areas familiar to the authors, such as the exposition of the rumen associations in Chapter 8 (which deals with symbioses in general).

A short book of such broad scope in so newly developing a scientific area cannot fail to generate disagreement, but the final paragraph of the authors' preface disarms criticism: "Readers may find . . . that it is biased in some places and that some topics are over-emphasized whereas others are treated superficially, or even contain misunderstandings". Amen. But the authors have certainly achieved the aim expressed in the sentence that follows: "We hope that the general principles of the subject will be understood". □

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